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ABBREVIATIONS

AFP	- α-fetoprotein)
ALT	- Alanine amino transferase
ANOVA	-Analysis were done by one way analysis of variance
ART	- Assisted reproductive technology
ASA	- Anti sperm antibodies
AST	- Aspartate amino transferase
AZF	- Azoospermia factor
BDL	- Below detection level
BPA	- Bisphenol A
BMI	- Body mass index
b. wt	- Body weight
CBAVD	- Congenital bilateral absence of the vas deferens
CKC	- *Chandrakanthi Chooranam*
CRF	- Case record form
DFI	- DNA fragmentation index
DL	- Detection limit
E2	- Estradiol
FSH	- Follicle stimulating harmone
GCP	- Good clinical practice
GIFT	- Gamete intrafallopian transfer
GSP	- *Gomutra silasathu parpam*
HCT	- Hematocrit
H & E	- Hematoxylin and Eosin

HPTLC	-High Performance Thin Layer Chromatography
HSP	-Human seminal plasma proteins
IAEC	-Institutional Animal Ethics Committee
ICH	-International Conference on Harmonisation
ICMR	-Indian Council for Medical Research
ICP	- Intracavernous pressures
ICP-OES	- Inductively coupled plasma atomic emission spectroscopy
ICSI	- Intracytoplasmic sperm injection
IEC	- Institutional Ethics committee
IM	- Immotility
i.p	- Intraperitonial
IUI	- Intrauterine inseminations
IVI	- In vitro fertilization
LH	- Luetinizing harmone
MAR	- Motile spermatozoa with bound particles
MCP	- Membrane cofactor protein
MDA	- Malondialdehyde
MSG	- Monosodium glutamate
NCLAS	-National Centre for Laboratory Animal Sciences
NIN	- National institute of Nutrition
NOAEL	- No observed adverse effect level
NP	- Non-progressive motility
OATS	- Oligoasthenoteratozoospermia
OPD	- Out patient department
OS	- Oxidative stress

PCT	- Platelet crit
PDW	- Platelet distribution width
PLIM	- Pharmacopoeial Laboratory for Indian Medicines
PLT	- Platelet count
p.o	- per oral
ppm	- Part per million
PR	- Progressive motility
PRL	- Prolactin
PTN	- Protodioscin
RDW	-Red blood cells width
SD	- Standard deviation
SE	- Standard Error
SEM	- Standard error of the mean
SHBG	- Sex hormone binding globulin
TD	- Therapeutic dose
TDS	- Testicular dysgenesis syndrome
TGA	- Thermo gravimetric analysis
TLC	- Thin Layer Chromatography
TPN	-Total Protein
TT	-Testosterone
v/v	- volume/ volume
w/w	- weight/weight
ZIFT	- Zygote intrafallopian transfer

1. INTRODUCTION

"Be fruitful and increase in number"this was the first blessing given by the Lord to the mankind.[1] Ever since the commencement of documentation of history, the human has positioned a great importance on fertility.[2] Reproduction, continuity, maintenance through the descendants and the desire for self protection forms the fundamental need of family unit. Infertility represents a severe emotional and common problem in the social order where importance is emotionally involved to have offspring.[3] 50 to 80 million general populations suffer due to infertility on world level. The World Health Organization (WHO) has estimated about 8 to 10% of couples goes through this problem.[4] Male part is exclusively accountable with reference to 20 % of unfruitful couples and contributory factor in 30 – 40%.[5] 50% couple infertity, is due to Oligospermia a male medical condition.[6] In the year 1981 Indian census estimated around 4 to 6% infertility. Survey on southern part of India as on 1993-2005 showed deterioration in sperm conut, motility and morphology.[4] Infertility is defined as the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy in one year".[7] WHO guiding principle reports, "Count less than 15million/ml is defined as oligozoospermia".[8]

Selection of the disease

In the present days male infertility is increasing due to factors like environment, alcoholism and smoking which lead to poor semen quality.[9] Carlsen et al. observed considerable decline in the mean value of sperm count from 113 million per ml (1940) to 66 million per ml (1990) and a drop of 0.94 million per ml for every year.[10] Infertility is usually the presenting illness and investigations might lead to the finding of oligozoospermia, asthenozoospermia, teratozoospermia and azoospermia. In medical practice, oligozoospermia is considered single most common cause for male infertility.[11] Investigator preferred in spot lighting male infertility however such an extensive issue cannot be studied in limited time and hence the study was limitied to be focused on Oligozoospermia.

Causes for Oligozoospermia

- Pretesticular cause which includes hypogonadism, smoking, drugs, alcohol, medication and strenuous riding.
- Testicular causes which include genetic defect, age, neoplasm, varicocele, hydrocele, cryptoorchidism, mumps, malaria and trauma.

- Post testicular causes which include lack/obstuction of vas deferens, obstruction of ejaculatory duct, genetic markers for cystic fibrosis and infections.[12]
- Obesity has been proposed to affect male fertility both directly and indirectly by inducing alteration in sleep, sexual behavior, hormonal profiles and scrotal temperature.[13]
- Environmental toxins like insecticides, pesticides and heavy metals highly afffect reproductive organs.[14]
- Since past decades distorted lifestyle, chemical based foods, lack of nutrition, pollution, tight clothing, deskbound work and stress have reduced fertility.[4]

Siddha Background [1, 15]

Siddha system of Medicine [SSM] is started off from Tamil Nadu, south state of India. Practiced generally in and around the areas of its foundation. The drug resources of SSM have been classified into three main groups, plant resources, inorganic resources and animal resources which are described by means of *suvai* (taste), *gunam* (quality), *veeriyam* (potency), *pirivu* (post-digestive taste) and *prabhavam* (specific action). Diagnosis of disease in *Siddha* system is based on the assessment of *envagi thaervu* (eight fold examination) which are tongue, colour of the body, voice, eyes, stools, urine, touch and pulse. Most prominent procedures used for diagnosis and prognosis of diseases are *neerkuri, neikuri* (urine examination) and *naadi* (pulse examination). *Siddha* system had already gained an insight in individualistic management through *thega ilakkanam* (biotype - characterization of an individuals) which reduces the possibility of misdiagnosis / incorrect treatments, which now contemporary research is testing to achieve through pharmacogenetics and pharmacogenomics. In *Siddha* science, 96 *thathuvam* (principles) deals with the basic components of human body which include intellectual, physiological, physical and psychological components of the individual.

SSM describes the human body is made of five primordial basic elements like space, air, fire, water and earth which are the building blocks of physical and subtle bodies. Siddha scientific principles, has been already present in the universe. The Lord God formed the man of dust [earth element] from the ground and breathed into his nostrils the breath of life [air-*vatham*] and the man became a living creature. According to Siddha medical science the element earth gives fine shape to the body, including bones, tissues, muscles, skin and hair. The physiological function in the human system is mediated by *uyir thathukkal* (functional

constitution of the body) i) *vatham* - air (bio energy movement) ii) *pitham*-bile (bio energy fire) iii) *Iyyam*-phlegm (bio energy water) which is formed by the combination of the five primordial basic elements. *Saaram* (chyle), *senneer* (blood), oon (muscle), kozhupu (cholesterol), enbu (bone), moolai (bone marrow), *sukkilam/suronitham* (semen/ovum) are *udal thathukkal* (physical constituents) which are identical to the various types of tissues. SSM defines disease as the signs and symptoms resulting from vitiation of intrinsic factors i.e three humors and seven physical constituents due to changes in extrinsic factors such as diet and activities. Drugs of SSM are used separately or in combination for maintaining the three humours

***Siddha* ethical guidelines and preventive measures for diseases [15]**

Indications for Preliminary treatments

Diseases are determined with the help of pulse reading. Clinical condition of the patients like diet habits, sleeping pattern, urine, motion and five sense organs should be assessed.

People more prone to the incidence of disease

People who are on irregular diet, sendentary life; consuming food without proper mastication; devoid of exposure to first sun light (morning), consuming food which make worse the *kabam*, impure water, inhaling the air from graveyards, intercourse with elder women, are more prone to the incidence of disease.

Preventive measures

Consuming nutritious food, inhaling pure air, drinking pure water**,** doing physical exercise**,** intercourse with younger women during night, not suppressing the natural urges (14 reflexes). Regular practicing of all these behavior can lead to a blissful life.

Comparison of Thathu nashtam to Oligozoospermia

According to Siddha Scholar *Yugi muni* symptoms like semen showing lack of sweetness, floating on water, absence of vitality & frothy micturation indicates *Aan maladu* [male infertility].[16] In *Siddha, Thathu nashtam* [Oligozoospermia] has not been described as a separate disease entity. There is no direct reference for the comparison of *Thathu nashtam* to oligozoospermia however its feature can be understood on the basis of the indirect references available in the Siddha literatures**.** The various nomenclature used in siddha texts in relation with *Sukkilam* the seventh *udal thathu* [physical constituent] can be compared to sperm and not semen alone since its function is reproducion. The term *Thathu Rogam [vinthu vai patria*

noi] denotes disease relating to sperm and *Thathu kuraivu / Thathu pushti kuraivu* denotes Oligozoospermia[17] and hence the term *Thathu nashtam* can be compared to oligozoospermia of contemporary science as both are similar in terminology [oligo-low; zoospermia-spermatozoa in semen ; *thathu* –sperm; *nashtam*-lack, be deficient in].

The empirical therapies

Male infertility is evaluated through investigation of semen and hormonal analysis (testosterone, follicle stimulating harmone and lutenizing harmone).[18]The empirical therapies in treating oligozoospermia includes androgens, gonadotropins and antiestrogens. Majority of these treatment put importance on the stimulatory effect of testosterone in spermatogenesis.[19] Fertility depends on synchronized functions of reproductive tract axis-hypothalamus, pituitary and gonads.[20] Alteration in life style, intake of balanced nutritional diet and supplements may increase sperm count and motility. Among micro nutrients Vit B12, Vit C, Vit E, carnitine, arginine, selenium and zinc play significant function in increasing sperm count and motility and each has definite role in increasing sperm count and improve its function.[14] Antioxidants may protect sperm DNA from oxidants/free radicals and may increase stability of blood testis barrier.[21] Intracytoplasmic sperm injection, ever since from its introduction (1992), has revolutionized the male infertility treatment.[20] In vitro fertilization and intracytoplasmic sperm injection provide symptomatic managements and the cause of the limited success may be the requirement of methods to identify ART (Assisted reproductive technology) type providing the most possible chances of pregnancy in a couple.[22]

Need of the hour

Inspite of revolutionary progression in treatment of infertility in the most recent decade through drugs, diagnostic procedures, and assisted reproductive technologies, the expenditure of ARTs is still extremely high and beyond the reach. Most part of infertile men do not need expensive and advanced trial.[3,23] In most of the times invasive technology (ART) creates economical and emotional stress and may not assure success. Alternative therapies (*Siddha*) should be developed to guarantee the wish of the infertile males to give forth to bilogical offspring in a natural way. Conversely scientific perspective documentation through clinical trials are required to assure the safety and efficacy of the drugs.[24]

Choice of the drug

- Siddha sastric herbomineral formulation *Chandrakanthi Chooranam (CKC)* consist of twenty five ingredients and is indicated for the treatment of oligozoospermia, vaginal diseases, veneral diseases, polyuria and in all biliousness.[25]
- Traditional utilization of the ingredients of *CKC* claims its function in treating male infertility, oligozoospermia, impotency, nocturnal emission, premature ejaculation, spermatorrhoea, high and low viscous semen, male gonorrhoea, testicle swelling and prostate enlargement.[24]
- Some ingredients in *CKC* act as an aphrodisiac drug, testosterone booster and also counteract alcohol intoxication.[24]
- In general, mineral medicines progress semen immediately. Spermatogenic activity of *Shilajit* (mineral) is reported in rats and in clinical trial of patients with oligozoospermia and there was significant improvement in the semen quality.[24]
- The composition in *CKC* is specifically judged and thus motivated the investigator to select it as the study drug to provide treatment for oligozoospermic patients.

***Siddha* claim in treatment of Oligozoospermia**

- Synergistic features are exceptional to phytotherapy and the part of the ingredients in this combination contributes both to efficacy and safety

- Shilajit is referred to thathuras (body tissue) and it is the rasayana that tonifies the action of the *saptha thathus* (seven body constituents) that is chyle, blood, muscle, fat, bone, bone marrow and reproductory fluids of the body (semen)

- Shilajit augments the bioavailability of the herbs in the body.

In this study the investigator have intended to establish both the safety and efficacy of the siddha herbo-mineral formulation *Chandrakanthi chooranam* to be proved in clinical and preclinical studies and to provide it as a standard treatment for oligozoospermic patients by improving sperm parameters.

2. AIM

- To evaluate the safety and efficacy of *Siddha* herbo mineral formulation *Chandrakanthi chooranam* through preclinical and clinial studies

OBJECTIVES

I] Preclinical

a) Standardisation of *Chandrakanthi chooranam*

- Identification and authentication of the herbal and mineral constituents
- Pharmacognostical study for the selected herbal constituents
- Preparation of the study drug
- Analytical specifications of *Chandrakanthi chooranam* (PLIM guidelines)
- Thermo gravimetric analysis (TGA)
- ICP-OES elemental analysis

b) Animal studies

i) Toxicity study (Safety study)

- Acute and long term toxicity studies to evaluate the safety of the study drug

ii) Pharmacological study (Efficacy study)

- Evaluation of spermatogenic activity of the study drug in ethanol induced sperm reduction in wistar rats.

2] Clinical Trial

A) Pilot study

- To assess the feasibility, safety and tolerability of administration of the study drug in Oligospermic patients prior to conducting phase II main clinical trial

B) Main clinical Trial

- To evaluate the changes in the sperm concentration, percentage of total and progressive motility and the percentage of normal forms of sperm per milliliter of seminal fluid
- To estimate the changes in serum Testosterone, FSH and LH harmones
- To assess the clinical safety parameters

3. LITERATURE REVIEW

3.1. SIDDHA LITERATURE REVIEW

DEFINITION [17]

Table 3.1.1: Siddha Definitions

Maladu **(Infertility)**	A disease by which men/woman are rendered incapable of producing child by reason of defective semen or menses in them is termed as *Maladu*
Aanmaladu **(Male Infertility)**	Want of fertility or fecundation in a man's semen
Sukilam **(Semen)**	Semen is formed by the essential parts of bone marrow the sixth constiuent of the body mixed with blood. It is the support of the body and the root of pregnacy.
Vinthu **(Sperm / Masculine embodiment)**	A term to denote the male reproductive cell / gamete / characters

Etymology for *Sukilam* [26]

Derived from tamil word, *Sukilathuvam* - venmai; *Sukilam* - venmai (white / purity)

Anatomical [*Angadhi padham*] and Physiological [*Sukaranam*] terms [17,27]

Table 3.1.2: Anatomical and Physiolgical terms in *Siddha*

Bijam, Lingam, Andam, Vithai (Seed)	This has the capacity to give rise to new offspring
Vinthu (sperm)	*Sukila kirumi* (spermatozoan); *Sukilathathu - vinthu*; *Sukilam - vinthu*
Sukila Vasayam	Repository [Testes and ovaries] for reproductive tissue
Manomaya kosam **(Mental system / psychic system)**	Constituted by the mind and the five organs of perception
Niyana Inthiriyam:	Action of genital organ
Mulatharam **(Neuro endocrinal centres)**	Located between the rectal and external genitalia [inside the perineum] at the base of the spinal column. Resting place of serpent power / rectal plexus
Swathittanam **(Neuro endocrinal centres)**	It is situated two finger breadths above the *moolatharam*. It is a constituent of earth element
Thi Mantalam **(Fire Zone)**	This is found two finger breadths above the *moolatharam*
Thinkal Manadalam **(Lunar zone)**	Lies in the centre of the eyebrows from where emerges brightness like that of millions of moons and stars
Kuntalini	A form of energy located in the *moolatharam*
Abana vayu	Component of vatham-downward air
Theka Ilakkanam **(Bio Type)**	The nature of an individual including structural, functional characteristics, adaptability, a relatively stable and genetically predetermined which is classified into four main types namely *Vali/ Azhal/Iyyam/ Thontham*

Synonyms for Oligozoospermia

- ***Thathu Rogam (Vinthu vai patria noi):*** Disease relating to sperm [17]
- ***Thathu kuraivu / Thathu pushti kuraivu***: Oligospermia [17]
- ***Vinthu kuraivu****:* Oligospermia [28]
- ***Vinthu anu kuraivu****:* Oligospermia [29]
- ***Sheena vinthu***: Oligospermia [17]

Other related terms

- ***Sukila nattam***: spermatorrhoea [17]
- ***Vinthu nashtam***: spermatorrhoea [17]

Epidemiology

Elavenil [Early summer] aggravates the disease [30]

Siddha **philosophy and physiology of sperm**

Fertility period (Ovulation days) [31]

As per saint *Agathiyar*, on the first day of women menstrual cycle her genitals bloom like a lotus having fourteen to sixteen petals. From the first day onwards till the fourteenth or sixteenth day each petal closes one by one every day; within these days if the *vindhu* [sperm] which is chiefly constituted by the fire and air elements, enters with the *natham* [ovum], then all the petals will immediatey closes. Thus if a man and woman copulates within the first fourteen or sixteen days of the menstrual period it is easy for formation of zygote to evolve stay in the uterus.

Acrosomal reaction of sperm and embryogenesis in Siddha [17, 32, 33]

"*Natha vintu vilungum vayu"* For gestation in union of the male and female fluid *vayu (pranan*) is needed. The simile of mixing together of *thaneer* (water) and *sunnabu* (calcium) is mentioned to explain the fusion of the ovum and sperm to develop into an embryo. This can be explained through the following research work done in sea urchin. The increase in intracellular Ca (calcium) will cause water to enter the cell, and will increase the hydrostatic pressure. This will support the extension of acrosomal reaction (calcium dependent reaction) of the sperm. Then the acrosome fuses with egg's plasma membrane (beneath the vitelline layer). The sperm head will now have the access to the cytoplasm. Sperm alone are not required for activation of the egg and the calcium can induce egg activation.

Elemental composition and three humour in human embryology [31]

The *natham* (ovum) consists of the element earth, whereas the *vinthu* (sperm) consist of fire and air. The uterine wall which nourishes it has water element and the uterine cavity is of the element space. Therefore in the formation of foetus all the five elements combine and create it. As per saint *Dhanvantari*, when the sperm and ovum combines on the very day three kinds of humor's spring forth.

Microscopic nature of sperm [32]

Vinthu and natham are parama anu. Saint *Yugi* says, *munai arugu nuni* ***pani pol*** *suronethathil sernthu thakavey*. The sperm and the ovum are very microscopic the invisible base of aggregate bodies, they are like a dew on the sharp tip of a grass which means the semen and ovum merges and that the sperms in the semen moves in utmost swiftness and joins.

Morphology of sperm [31]

As per saint *Thirumoolar***,** the semen containing innumerable spermatazoa passes very swiftly (motility) in to the uterus with rapid vibration of their tail. It is understood that the sperm has tail.

Sperm Motility [32]

From the poems, *vayu odu vinthu* ***senru*** *malarkul sernthal*; *pinumam sukilathil piranavayu than* ***selum*** and *vinthu anga* ***oorum*** *pothu*, it is explained that with the help of *vayu* (kinetic force- pranan) sperm move in to uterus and fuse with ovum.

Functional sperm [32]

As per saint *Yugi, Vinthu thayin karbathil vegu thanthiramaga selum,* ***Oru pani thuli alavey nilaithu nirkum*****.** These lines denotes that only one functional sperm is necessary for fertilization.

Preventing polspermy [32]

From the poem, *Vayu odu vinthu senru malarkul sernthal,* ***malar ethel-yelam moodi kolum*** *and Veli pol* ***valinthu kakum*** *vinthu udan prana vayu,* it is explained that abana stays outside and the pranan goes along with the spermatazoa and bisects the size of the zygote. Just as the fence guards the garden the air surrounds the *thiranda suronitham* (zygote) and

guards it and prevents other sperms entering it. (preventing polyspermy). Although many sperm attach to the coats surrounding the egg, it is important that only one sperm fuses with the egg plasma membrane and delivers its nucleus into the egg.

Aperture [32]

Saint *Yugi* says, *munai arugu nuni pani pol suronethathil serunthu thakavey* and *vaithya saaram* says, *paintha veli thamar pol*. The spermatozoa enters by penetrating the wall of the ovum by penetrating it and where the sperm enters in to the ovum is perforated with apertures.

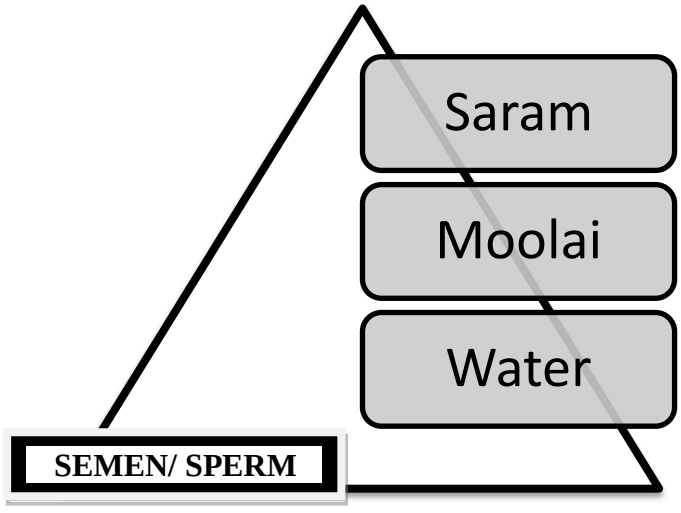
Sex determination [31, 32]

As per *Athuva Thathuvam*, the fusion of the *sukkilam* and *suronitham* constitutes the human body. The variations can lead to deformities. Depends upon the dominance of *sukkilam* or *suronitham* the male or female birth take place. At the time of copulation if the male dominates then it is male and if the female dominates then it is a female. If the male and female are equal then the child will be neutral gender or a eunch. Here male indicates the *vindhu* and the female indicates *nadham.*

Formation of the semen/sperm

i) Formed from *Saaram*
ii) Formed from *Moolai Thathu*
iii) Formed out of Water eleme

Figure 3.1.1: Formation of the semen/sperm



Formed from *Saaram* (Chyle) [32]

According to *saint Theraiyar* the concentrate of the food becomes, *saaram* (chyle) the same day; *Senneer* (blood) the second day; *Oon* (muscle) the third day; *Kozhupu* (cholesterol) the fourth day; *Enbu* (bone) the fifth day; *Moolai* (bone marrow) the sixth day and *Sukilam/Suronitha* (sperm/ovum) the seventh day. From chyle the essentials were taken up to semen and were produced. *Saaram* (chyle), *senner* (blood) *and venneer* (sukilam) one day fuse to form *vinthu* (sperm)

Formed from *Moolai thathu* (bone marrow) [34]

Modern research showed early stage sperm cells had been created from bone marrow *(moolai)*. Stem cells were isolated from bone marrow of donors (male) and.then cultured and identified in the laboratory. Some were induced to grow into spermatological cells, which generally become sperm cells. Transplantation of cells inside the testis was carried out in mice.

Formed out of water element [31]

The physical component of the water element are blood, fat, semen, urine and brain. *Iyyam* (bio energy water) one of the three humour is condensed from the elements water and earth and it lies in sperm, fat, blood, bone and bone marrow. Measurement of semen (water component of the body) is ½ *anjali* (two palms held together to shape a bowel like structure and its capacity is called one *anjali*)

Spermatogenesis [35]

Table 3.1.3: Spermatogenesis in Siddha

80 drops of blood makes 1 drop of semen
80 drops of semen makes 1 sperm
6400 drops of blood makes 1 sperm
1000 drops of blood + 3000 drops of blood and semen makes 1 sperm

Spermiogenesis [35]

Saaram (chyle), *senner* (blood) *and venneer* (sukilam) one day fuse to form *vinthu* (sperm) which grows in body for 21 days (*yel mundru thenam)* which can be correlated to the differentiation of spermatids to mature sperms

Nutrition [32, 27]

Vani (fire) gives nutrition to sperm. For processing saram to sukilam *agni* (basal metabolic heat-*samakini*) is needed. Testosterone, FSH and LH and are responsible for stimulation of spermatogenesis process. Stimulation also needs *agni*. *Agni (Vani, Azhal)* is formed from the element fire and is the principle of transformation energy and governs heat and metabolism in the body and is concerned with the digestive, enzymatic and endocrine systems.

***Sukila Gunam* (Physical Characteristics)** [32,16,36]

Table 3.1.4: *Sukila Gunam*

State	Water
Colour	White and butter –Excellent White and curd -Very Good White and milk- Good
Specific gravity	Should sink in water
Taste	*Innipu* (Fructose)
Chief elemental composition	Fire and water
Predominant humour	*Aiyam*

Saint *Sivavakiyar* says, ***Unmayana sukila ubayama erunthathum, venmayagi neeriley virainthu neera thanathum.*** Semen ejaculated is milky white in appearance due to prostatic secretion. Immediately after ejaculation, sperm remains immotile for the reason it is viscous due to coagulation and becomes highly motile as the coagulation dissolves.

The characters relating to sukilam will be comparatively better in *iyya thegi* individual due to similarities of gunam.

Function of the *Sukilam* [32]

Responsible for reproduction.

Harmone [36]

Saint *Thiruvalluvar gana vettiyan* says, ***Vinthu kudi iruntha thirunattai vitaen, marukinra kathirikol pattathanil, vinthunindru vilangunathi mayathuley, vilangu swathittanam veliyilethan.*** *Swathittanam* can be correlated to adrenal gland that secretes testosterone.

Siddha Physiology [32, 36]

Secretion of semen may be controlled by *moolatharam* (resting place of serpent power/rectal plexus), *manomaya kosam* (pschyic system), *swathithanam* (psycho physical center above *moolatahram*) and *iyyam (water).*

As per saint *Thirumoolar, uthayathil vinthuvil ongum kuntalium*. *Kuntalini* (serpent form of energy) facilitate the act of emission of semen. Ejaculation is also controlled by *kanma-inthiriyam* (motor organs), *abana vayu* (downward air) and *sadhaka pitham* (accomplishment fire)

Uyir thathukal **(Bio energetic principle)** [27, 32, 37]

Vali **(Bio energy air)**

It is located in *idakalai, abanan, kama kodi* (spermatic cord). *Vali* is the principle of kinetic energy in the body and is concerned with the nervous system and with circulation. It activates andcoordinates the seven *udal kattugal* (physical constituents). *Abanan* one of the component of *vatham* is the downward air responsible for excretion of semen

Azhal **(Bio energy fire)**

The heat of *azhal* is responsible for many actions. *Azhal* lies in blood and chyle. *Sadhaka Pitham* seems to have the psycho-somatic role in the formation of semen and human desires. *Azhal* is the principle of transformation [conversion] energy and governs heat and metabolism and is concerned with the digestive, enzymatic and endocrine systems.

Iyyam **(Bio energy water)**

It lies in semen, fat, blood, bone and bone marrow. *Iyyam* is the principle of stablizing energy and governs growth in the body and is concerned with structure, stability, lubrication and fluid balance. *Tarpaka Kabam* [CSF fluid] has important role in production of *sukilam*. Major component of human semen is cerebro spinal fluid.

Noy Muthanatal **(Aetilogy)**

Genetic cause: ***Theka Ilakkanam*** **(Bio Type)** [32, 36]

- *Vali* (ectomorphic constitution) - *Thatu nashtam* (Oligozoospermia)
- *Pitham* (mesomorphic constitution) - *Arpa sukilam* (Oligozoospermia)
- *Aiya* (endomorphic constitution) - *Inthriya kuraivu* (Oligozoospermia)

Infections [17, 32]

- As per saint *Thirumoolar*, ***Varaiyana garbathil malatu pulu pugil***, ***viraiyana vinthuvai virainthun didumel*.** Sperm is eaten up by *maltu pulu* (may be antisperm antibodies) in uterus.
- *Sukila piramium/megam* (male gonorrhoea)
- *Koruku noi* (Syphilis)

Testicular cause [32, 36]

- *Beeja Thamba Vatham / Neerandam* (Hydrocele)
- *Asuva vatham* (Crypto orchidism)
- *Vithai Vatham* (Orchitis)
- *Sukila Pitham* (Varicocele)

As per *Dhanvantari vaithiyam, Thakathan karbam thanai thavirthidum, kanavu thanil sakidium sukilathai sukila nalam kanthum, mika sukilam polneeril vellaiyum kannum* ***kandal***, ***sukila pitham*** *menrai solinar sruthivallor*. Here *sukila nalam kanthum* refers to burning sensation in ejaculatory ducts.

Traumatic causes [38]

- Surgery or accident sometimes leads to *varmam* (vital points / subtle energy station)
- *Kallitaikkalam / Vithu Varmam*
- *Beeja kaalam*- Testis will be found ascended through the inguinal canal

Psychiatric disease [38]

In *Mathazhivu / Mathathiam udal thathus* (physical constituents) including *sukilam* will be affected.

Taste [39]

Table 3.1.5- Consequences of affected Taste

Increased *Pulipu* (sour)	Weakness of genital organ
Increased *kaippu* (bitter)	Abnorml change in semen qualities
Increased*karppu* (pungent)	Oligozoospermia
Decreased *innipu* (sweet)	Lack of maintainence of seven body tissues

Dietary factors [30, 40]

Macrotyloma uniflorum, yam (karunai kizhangu), mango (unripe), bitter gourd, sesban leaves, lily pond water and red water.

Life style [30, 35, 36]

Intercourse with elder female, restraining ejaculation (one of the 14 functional natural urge) wearing slippers made of *Acacia catechu* (*karungali*), *Cedrus deodara* (*devadaru*), *Wrightia tinctoria* (*vetpalai*), *Morinda citrifolia* **(***nuna*) and *Ficus religiosa* (*aal*)

Gonado toxic drugs [40]

Nicotiana tabacum

Diurnal factors [36]

Intercourse during digestion of food, intercourse in day time [intercourse at improper time] and intercourse during sunset

Oligozoospermia coexpress with the following diseases [41]

Table 3.1.6: Diseases that coexpress with Oligozoospermia

Perumanjal kaamalai (Jaundice)
Eraippu (Bronchial asthma)
Vinthu Suram and *vali azhal suram* (Fever)
Aiya Pandu (Anaemia)
Mukutra Kalladaippu (Urolithiasis)
Vali Neer kiricharam (Urinary tract infection)
Neerazhivu avathai (Diabetic complication) – In second stage *sukilam ketu* (sperm destruction) and *oli kunrum* and fifth stage will cause *vinthu nashtam* (Oligospermia).
Elaippu Noi (Pulmonary Tuberculosis)
Eruvaaimulai Noi (Haemorrhoids)
Karumpa nasai ammai (Measles) - Affect semen and can make the patient *maladu*.

Pathalogical state of semen

Table 3.1.7: Signs of semen in Male Infertility as per saint *Yugi* [16, 42, 43]

Devoid of sweetness [Absence of fructose]	Normal value of seminal fructose is > 13 µmol/ejaculate (WHO 2010)
Floating on water [decreased semen specific gravity]	Normal specific gravity of semen is between 1.020 and 1.040 which is higher than that of water and when it reduces its floats which is the sign of infertlity
Absence of live sperms [Absence of vitality]	Normal value of live spermatozoa is 58% (WHO 2010)
Foamy urine	Foamy urine is most likely to appear in case of urinary tract infections and retrograde ejaculation, a condition in which the sperm reaches the urinary bladder

- Increased *sukkilam* causes increased lust, libido and urinary calculi.[27]
- Decreased *sukkilam* causes failure in reproduction, pain in genitalia, dropy ejaculation of semen/ or blood during copulation, pricking pain in the testis, inflammation and blackening of genitalia.[27]
- Semen if restrained results in fever, retention of urine, joints pain, heaviness of the body, heart diseases and white discharge. [27]

Pathogenesis [27, 30, 32, 39, 40, 41, 44]

- *Three humours* play a major role in the initiation of the disease. Vitiated *vatham, pitham* and *iyyam thodam* consecutively vitiate *sukilam* (semen/sperm) and decreases it either quantitatively or qualitatively
- Mental stress and strain reduces sperm count in which *vatham* and *saram* is deranged (air element has the property of mental agony, *saram* gives mental and physical perseverance)
- In repeated intercourse since fire element is affected, sperm production is decreased. (fire element has the property of sexual intercourse***)***
- In excessive sexual abstinence, the activity of *vayu* is dreanged and sperm motility is affected.(*vali* is the principle of kinetic energy)
- Even if *saaram* is well nourished and absorbed normally, it should be available up to *sukilam*. In excessive physical strains, most of the *saaram* is utilized and does not reaches till *sukilam*.
- Intercourse with elder female causes *iyyam* derangement
- In Testicular atrophy *vali* is deranged (abnormal functions of deranged *vali* is weakness of functional organs) and decreases spermatogenesis.

- In inflammations (orchitis,prostatis), mumps, jaundice, urinary tract infection, seminal infections and fever *pitham* is deranged.

- The maturation arrest in spermatogenesis can also be considered as *pitham* derangement since *azhal* is the principle of transformation [conversion] energy.

- In crypto orchidism *pitham* is deranged which affects (temperature difference) the testis.

- Obstructive pathology (ejaculatory duct) can be considered as *iyyam* derangment (due to properties of solidity, nature of immobility]

.

- Alcohol is *thamo gunam* (*vatha*) food articles and hence derangment of *vali* causes weakness of functional organs.

- *Nicotiana tabacum* (Tobacco,smoking) affects pitham and destroys sperm (*vinthualikum*)

Table 3.1.8: Deranged three humours and affected semen quality

Sperm production (Oligozoospermia) is affected by *kabam* derangment (*iyyam* lies in semen)
Sperm motility (Asthenozoospermia) is affeted due to *vatha* derangement (kinetic force)
Sperm morphology (Teratozoospermia) is affected due to *iyyam* derangment (governs growth, concerned with structure).
Azoospermia (Obstructive pathalogy) shows absence of fructose (*innipu taste*) in semen due to *iyyam* derangement (innipu - water + earth = kabam)
In Necrospermia *vatham* (kinetic energy) is decreased, *iyyam* (immobility) is increased
Low viscosity is due to *vatham* derangment (dryness) and high viscosity is due to *iyyam* derangment (solidity, fluid balance is the property of *iyyam*)
Semen liquification is affected due to *iyyam* derangement (fluid balance).
Semen volume is affected due to *iyyam* derangment (*iyyam* - water and earth element)

- Frothy micturation urationis due to *iyyam* derangement (*iyyam* is eliminated through urine

- Floating on water is due to *vatham* derangement which has the property of lightness.

- *Uyirupu illathathu* (Necrospermia- absence of live spermatozoa) due to *vatham* (kinetic force) and *iyyam* (immobility) derangment.

- Pungent (*karpu* - air and fire) and bitter (*kaipu*- air and space) taste causes *sukila kedu* (destruction of sperm production) due to derangment of *pitham* (fire) and *vatham* (air)

- *Saaram* is impaired due to *deekshagni*- (fiery digestion) in which due to increased digestive fire food gets digested along with essence and *manthagni* in which food is digested delayed. The affected *saaram* affects sukilam.

- When *pitham* (transformation energy) is affected, spermatogenesis (primary spermatocytes to immature sperm) and spermiogenesis (immature to mature sperm) will be affected

- *Iyyam* derangement increases viscosity of semen which indicates infection in the genital tract or the presence of antisperm antibodies. Viscous interfer's with determination of motility and antibody coating of spermatozoa.Vitiated *iyyam* condensed with the element earth, has the propety of conglomeration, solidity and viscuous which leads to the formation of agglutination of sperms due to inceased viscosity of semen, which hampers the motility of sperms and results in asthenozoospermia

- As *moolatharam* is in the *akkini mandalam*, any pathological condition here can harm the *moolakini* and eventually derange the *pitha* humour. Symptoms are produced when deranged *pitham* affect the seven thathus.

- The disturbances in *seeva agni / pitham* [basal metabolic heat] due to food articles and behaviour like sour, pungent, salt taste; unboiled food; sorrow; hot temperature, angry, insomnia and excessive coitus leads to *vishamagni* (toxic digestive fire).In which digestion is delayed due to deranged and displaced *samanan* (digestive air) which leads to toxic digestion and which will produce *amam* (indigested food toxin). *Amam* is the toxic material produced from undigested food and is a disease connected with the mucous (viscuos fluid-*iyyam*) in the intestines. *Iyyam* gets vitiated by *amam* [

free radicals, reactive oxygen species] which is responsible for improper production of rasa thatu which finally results in improper nourishment of sukila thatu

Associated symptoms [32, 41]

Ejaculatory dysfunction - *Vali (abanan)* is deranged in ejaculatory dysfunction. It is the downward air and responsible for excretion of semen.

Erectyle dysfunction - *Vali* is deranged in erectyle dysfunction. Vali is concerned with the nervous system and with circulation.

Nocturnal emmision - *Pitham* is deranged in nocturnal emmision. One of the symptoms of *sukila pitham* is, *kanavu thanil sakidium sukilathai.*

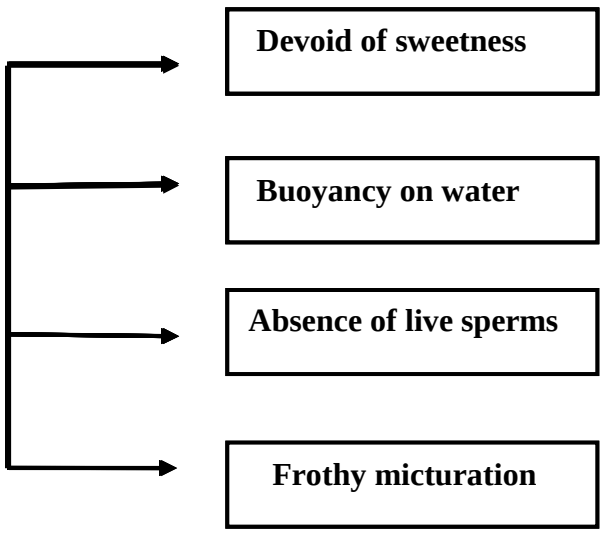
Noi kanippu vivaadham **(Differential diagnosis)** [32,41]

- *Sukila vatham* (Asthenozoospermia**)**
- *Sukila Megam* **(** *Pitham* type of *Neerizhivu* (diabetes) **)**
- *Sukila piramiyam / premegam* (Veneral disease)

Siddha maruthuva noi kanippu muraigal **(Diagnostic methodology)** [27, 32, 16]

- ***Neykkuri*** **(Oil on urine sign)-** Slow dispersal/formation of sieve/ flower/ animal/ irregular margin
- ***Nadi*** **(Pulse) -** vali naadi, vali azhal naadi, azhal vali naadi, pithathil vayu
- ***Manikadai*** **(wrist circumference sign) -** 8 ½ inch
- ***Sukila thervu*** **(Semen Analysis)**

Figure 3.1.2: Sukila thervu in Aan maladu



***Noi nidhanam* (Prognosis)** [32,45]

Table 3.1.9: Indication of Semen colour in Prognosis

Colour	Prognosis
White and butter like	Excellent
White and curd like	Very Good
White and milk like	Good
White and akin to buttermilk	Fair
Akin to the honey in colour and consistency	Average
Akin to the ghee in colour and weight	Poor
Akin to the toddy in colour thickness	Very poor
Akin to the water	Bad

Excellent, very good, good –can be controlled through medicine. Other types are difficult but can be normalized by medicine that increases the quality of semen

- **Neikuri** – Saladai kan (sieve) - Curable as per saint *Theriyaar* and intractable as per saint *Gowthamar.*
- **Manikadai-** 8 ½ -curable

Treatment

***Karpa marunthu* (Rejuvenation)** [27, 38]

- ***Karpa Yogam*:** *Vajira asana, Pranayamam, Yoga muthirai, Sarvaangaasana*
- ***Thathu karpam***- *Poorna Chandrothayam*
- ***Seeva Karpam***- *Inthira Koba poochi*
- ***Siddha* Panacea** –*Muppu* [Supreme Salt]- Normalize the body constituents

Medicinal Water [30, 31]

***Thathuundakum* (Spermatogenesis)**

Jambolana treee roots soaked water, cooked rice water kept over night, hot water kept in a gold pitcher and in iron pitcher, rain water before it falls down on earth and hot water drink.

***Thathu nattam* (Oligozoospermia)**

Yamuna river water, thungabhadra river water, sindhu river water and vaigai river water

Herbs and Animal Product [30, 40]

Greens of drumstick, climbing brinjal, spinash, *Amaranthus tritis,* wheat; plantain flower; coriander leaf, black plum; pomegranate; mango fruit, black grapes, cashew, almond and walnut, elephant milk; fresh milk (cow's milk) for 3 ¾ *naligai* (5hrs), milk skin or

lactoderm; mixed ghee (bufallo, goat and cow); thamboolam (conventional betel chewing), meat of domestic pig (*Oor panri*); black bug stag (*Kalai man*); meat of duck (*Vathu*); white breasted kingfisher (*Vichuli*); Ruddy Shelduck (*Thara kari*); spotted dove (*Mani pura*); goat meat (*Velladu*), Sea fish like emperor (*Valai*), eel (*Vilangu*) and ray fish (*Thiru kai*),

Season [6]

Pinpanikalam (late winter season) induces spermatogensis

Noi anuga vithi olukkam **(Preventive Techniques)** [27, 36, 41]

- ***Pariyanga yogam*** **(Spermatoschesis)**: Siddha art of sexual intercourse in which the *kama vayu (Pranan*) does not act and does not emmit semen. *Bogam* [sexual desire] was prefered in *Thi Mantalam* but the act was performed in *Thinkal Manadalam*.
- Coitus during day time is not advised.
- Avoid sexual act with elder lady (increases iyyam)
- Ideal time interval for coitus is once in a month
- The coitus performed during the time of digestion (after a full meal) should be avoided . *Saram* may not be completely nourished.
- During Sunset avoid coitus

Duration of treatment [31]

Every disease has a fixed period of treatment for complete recovery. For oligo spermia (*Thathu nattam*), the duration of treatment is one year.

Physician's Fees Prescribed [31]

The fees are also prescribed for diseases and it is clearly mentioned that treatment should not be given for ailments unless the fees is paid. The patient is morally obliged to pay the physician whatever he asks as his fees according to the set of the norms of the noble profession and for *Thathu nattam* (Oligospermia) it is 10 gold coins.

3.2. DISEASE REVIEW

DEFINITION [46,47,48]

- **Infertility (clinical definition):** "A disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse."
- **"Infertility** is regarded as 'male factor' when an alteration in sperm concentration and/or motility and/or morphology is present in at least one sample of two sperm analyses, which comply with the World Health Organization (WHO) 1999 guidelines, collected between one to four weeks apart."
- "A male who do not have biological offspring and presents for reproductive evaluation is labeled as "**primary infertility**," and one who is incapable to impregnate his partner but already has biological children is defined as "**secondary infertility**"

Table 3.2.1.Nomenclature related to semen quality [49]

Oligozoospermia	Total number of sperm below 15 $\times 10^6$ million / ml
Asthenozoospermia	Percentage of PR (progressively motile) sperm below 32 ml
Teratozoospermia	Percentage of normal morphological sperm below 4%
Aspermia	No semen (or retrograde ejaculation)
Azoospermia	No sperm in the ejaculate
Cryptozoospermia	Sperm absent in fresh preparations however observed in centrifuged pellet
Haemospermia	Presence of erythrocyte in ejaculate
Leukospermia	Presence of leukocyte in ejaculate beyond the threshold value
Necrozoospermia	Low percentage of live and high percentage of immotile sperm

ANATOMY AND PHYSIOLOGY

Reproductive organs [3]

The principal reproductive organs / gonads of the males are the testis, which produce sperm and as well secrete testosterone the male sex hormone. Prostate gland, seminal vesicles and bulbourethral glands are the accessory secretory glands which are involved in the secretion of seminal fluid.

Spermatozoa [3]

Spermatozoa are formed in the seminiferous tubules of the testis. Seminiferous tubules have many germ cells - spermatogenic cells and majority are in various stage of division. The outer most layer of spermatogenesis is in contact with the membrane which

surrounds each seminiferous tubule and these are termed as spermatogonia (undifferentiated germ cells) which divides mitotically and provides source of germ cells constantly. Spermatogonia that move about from the membrane will increase distinctly in size. These large cells are termed as primary spermatocyte which undergoes meiotic division and form 2 secondary spermatocytes, which consecutively divide in to 2 spermatids. Spermatids ultimately transform into spermatozoa (sperm). Semen in general contains about hundred million sperms per milliliters, even if it takes one sperm only to fertilize an ovum. Length of the normal spermatozoa is about 50-70 μm. Head being oval shape with acrosomal cap which measures about 3 to 5 × 2 to 3 μm, a middle piece (short) and a thin long tail (length which is about 45 μm).

Semen [3]

Semen is composed of fluids from the seminal vesicles, vas deferens, prostate gland and bulbourethral glands (mucous glands). Major bulk of the fluid is from seminal vesicle (60 %) which is last to ejaculate and also serves to wash the spermatozoa out of the ejaculatory duct and urethra. The protein concentration in seminal plasma is 35 to 55 mg/ml.

Sperm Quality and Semen Quality [50]

Sperms go throughout the ejaculatory ducts and mix with the fluids. Fructose secreted by the seminal vesicle is specially rich in semen and provide nutritonal energy to the spermatozoa. Seminal plasma is fundamental since it provides protective and nutritive environment for sperm to survive, mainly during the passage through the reproductive tract of female which direct to the fertilization. Most mammalian seminal plasma and sperm are greatly rich in zinc which is derived from the prostate. In addition to fructose, semen contains high level of Ca (calcium), Mg (magnesium), and Cu (copper) and they are bounded in ionic form.

To take in to concern six main criteria (Sperm volume, concentration, vitality, motility, morphology, and pH) as defined by WHO (world health organization – 2010) in grouping a semen into normal / subnormal.

Table 3.2.2: Lower reference limits for normal semen (fifth centiles and 95% confidence intervals) [49]

Volume	1.5 ml
Concentration	>39million sperms/ejaculate > 15 million/ ml of semen
Vitality	> 58 %
Progressive motility **Total motility**	> 32% > 40%
Morphologically normal forms	> 4%
pH	> 7.2
Peroxidase positive leukocytes	(106/ mL) < 1.0
MAR test (motile spermatozoa with bound particles	< 50 %
Immunobead test (motile spermatozoa with bound beads	< 50 %
Zinc	> 2.4 (µmol per ejaculate)
Fructose	> 13 (µmol per ejaculate)
Neutral glucosidase	> 20 (mU per ejaculate)

Endocrine Interplay [18]

The complete and successful germ cell (male) growth depends on the balanced endocrine interaction of hypothalamus – pituitary - testis. Hypothalmus secretes gonadotropin releasing hormone (Gnrh) and bring forth the release of gonadotrophins FSH and LH from pituitary gland.

- FSH bind with receptors present in sertoli cells and will stimulate spermatogenesis.
- LH will stimulate the steroidogenesis (testosterone production) in leydig cells which will act on sertoli cells and peritubular cells in seminiferous tubules and will stimulate spermatogenesis.
- Failure of pituitary in secreting FSH and LH may result in disturbance in testicle function which will lead to infertility.
- Testosterone, inhibin and estradiol will control the gonadotrophins secretion through feedback mechanism.

Primary hypogonadism is the disorder which directly affects the gonads. Secondary hypogonadism is due to defective secretion of pituitary gonadotropin. Testosterone, FSH and LH are the principal regulators of germ cell growth. Quantitative production of sperm generally needs the presence of these principal regulators. FSH directly acts on the seminiferous tubules while LH indirectly stimulate spermatogenesis via testosterone.

FSH acts a key role in the stimulation of meiotic and mitotic DNA production in spermatogonia. Androgen receptors are sited on sertoli cells and peritubular myoid cells. The signal has to be transduced by these cells, mainly in sertoli cells, since these receptors are not expressed on germ cells.

Spermatogenesis [50]

Spermatogenesis is exactly an organized development by which diploid cells transform into spermatozoa which take place inside the seminiferous tubules. Three distinctive phases may be divided in the development of spermatogenesis and they are

- **Profileration** - Increase in diploid spermatogonia;
- **Meiosis** - Spermatocytes into haploid spermatids
- **Differentiation** - Round haploid spermatids are differentiated into elongated sperm with mid piece and tail at final stage of spermiogenesis

Table 3.2.3. Micronutrients function during Spermatogenesis [50]

Calcium	Its important in sperm motility, acrosomal reaction and metabolism
Magnesium	Found in high concentration in the prostrate and is necessary for correct ejaculation
Potassiumand sodium	Have great role in acrosome reactions
Zinc	Involves in the ribonuclease activity and is highly active in the mitosis of spermatogonia and in meiosis of spermatocytes
Selenium [Antioxidant]	The high Selenium concentration in spermatogenesis is linked to its protective properties and to its related enzymes, like mitochondrial capsule protein in spermatozoa. If the Selenium content in seleno-proteins is low it likely decreases the chance of fertilization.
Folate	Folate has the antioxidant property which possibly inhibit apoptosis that results after DNA oxidative damage in spermatozoa
Nickel	Deficiency reduces the production of sperm in testis, count in epididymis and motility
Manganese	Manganese is suggested as the stimulator of pubertal growth

Table 3.2.4. Functions of Vitamins during Spermatogenesis [50]

Vit B12	Involves in DNA and RNA synthesis and will promote healthy development of seminiferous tubule
Vit B9	Promotes healthy spermatozoa and seminiferous tubule growth
Vit A	Differentiation of spermatogonia and in spermatid regulation
Vit C	Protects spermatozoa from the oxidative stress
Vit E	Improves the mitochondrial function

3.2.5. Biomolecules in Sperm Function [50, 51]

Lipids	Maintains sperm maturity, viability, function and fertility
Arginine	Precursor in the production of spermine, spermidine, putrescine and necessary in sperm motility
L-carnitine	Progression of sperm development, maturation and the maintenance of the quality
Tyrosine	Scavanges free radicals and improve the motility
Hyaluronan	Its the main protein derivative found to be in reproductive fluids and is involved in sperm motility and penetration
HSP-A1/A2 & A3	Concerned in the management of sperm motility and in sperm-oocyte binding
MCP- CD46	It is the complement regulatory protein and have role in protecting sperm from lysis in female reproductive tract and in interaction of sperm and oocyte
Neurotrophins (group of protein)	Plays role in the spermatogenesis and in the post- ejaculatory functions like motility, capacitation and acrosomal-exocytosis
Cholesterol	Secreted from the prostrate gland and is important in the protection of the sperm membrane integrity from environmental shocks by the chemicals/pollutants

Functions of Sertoli Cells and Leydig Cells [50]

Sertoli cells, specifically nurse cell, lines the inner walls of the tubules and participate partially in the differentiation process and provide nutritional and structural support for developing germinative cells. Secretion of the sertoli cells facilitates the transportation of non-motile sperm from testis in to the efferent duct. Leydig cells (interstitial cells) are interspersed within the seminiferous tubule which is the main site of steriodogenesis that produce testosterone in testes and also play vital role in the maturation of sperm.

Capacitation and Fertilization [50]

Spermatozoa have to be activated priorly to obtain the competence to fertilize the oocyte and this process of activation is defined as capacitation. The eventual organization of capacitation is within sperm membrane. Glycocalyx the outer surface of the membrane undergoes various biochemical alterations.Preprogrammed cellular derepression takes place which hyperactivate the sperm. The alterations include modifications in lipid and protein composition. Oviduct the microenvironment provides higher levels of bicarbonate than the epididymis and permits sperm capacitation. The capacitation needs electrolytes, metabolic energy sources (Ca2+) and protein sources like BSA (bovine serum albumin). To achieve fertilization perfectly and efficiently, it is essential for sperm to be progressive motile and the membranes should be intact.

AETIOLOGY

Y-chromosome micro-deletions [51]

Microdeletions in long arm of Y chromosome leads in to failure of spermatogenesis and ejaculation.Paternal lineage Y chromosome are related with low count and motility. Deletions of the regions that underline the heterochromatin in Y chromosome lead to the morphological abnormalities.

Chromosomal abnormalities [51]

Klinfelter's Syndrome is the common genetic abnormality found to be in 5% to 10% oligozoospermia peoples and is associated with failure of testicle and low count.

Anti-sperm antibodies (ASA) [51]

Anti-Sperm Antibodies are small proteins identified to deteriorate the fertility and semen quality through reducing acrosomal reaction, sperm motility, lysis of sperm, inhibiting the sperm penetration in to the cervical mucus and its capacitation.

Hormonal Disruption and Hormonal Imbalance [4]

- **Testicular dysgenesis syndrome (TDS) -** Congenital derangement in seminiferous tubulule structure and its function inextricably associated to inappropriate concentration of hormones (sex) at various stages of the life cycle which leads to male infertility.

- Testosterone deficiency will lead to the clinical condition hypogonadism. Abnormalities like reduced production of sperm and inhibition in the capability in fertilization take place in the male reproductive structure due to unbalanced action of the androgen during maturity.

- Copious amount of the circulating estrogens will suppress spermatogenesis and will adversely have an effect on male fertility.

Table 3.2.6. Anatomical Abnormalities in male reproductive system [4, 52]

Cryptorchidism	The testicles fails to descend into the scrotal sacs prior to birth.Abdominal testicles are not capable to maintain spermatogenesis since it needs the temperature less (2ºC) than the normal temperature of human for maturation of the spermatozoa to be functional, viable and fertilizable.
Varicocele	Collection of unusually swelled, dilated spermatic veins which drains the testicle. It may arise on both the side and most frequently on the left side. Varicocele will lower sperm quantity and quality and even shrinkage of the testicles.
Congenital anomalies	May be either rare with the localized defect in the vas deferens (proximal part) or with an complete abnormal growth
Epispadias	It is a congenital defect with abnormal curvature, shorter and wider size (penis) which will make the intercourse difficult.
Anomalies of the seminal vesicles	Abnormalities in number (fusion and agenesis), maturation (hypoplasia) and canalization (cyst)
Hypospadias	Uro-genital birth defect with abnormal urethral opening and it's the part of TDS which includes male infertility.

Alcoholism, Smoking [4]

Heavy and chronic alcohol toxication will have slow and progressive harmful impact. Will lead to moderate teratozoospermia which will be followed by the oligoasthenoteratospermia (OATS), then severe cryptozoospermia and finally azoospermia. Smoking and the passive inhalation of cigarette smoking will reduce the spermatozoa count, morphology, motility, viability and fertilizing capacity by increasing the seminal-oxidative stress and DNA damage. Nicotine, the major constituent in smoke has the considerable impact on morphology and count. Burning up of more than twenty cigarettes per day showed elevation in seminal Cd (cadmium) level in smokers.

Club Drug [4]

MDMA (Ecasty), GHB (gamma-hydroxybutyrate), methamphetamine, ketamine, morphine, heroin, marijuana, rohypnol, caffeine, cocaine and poppers. These addictions are accountable for the deleterious effect on complete sperm structure. Chronic addiction to cocaine has deleterious impact on spermatogenesis and eventually fertility.

Lifestyle [4, 53, 54]

Decline in fertility with the age is related with a decrease in the testicular weight, spermatozoa production and level of testosterone. Obesity and overweight will end in hypogonadism, higher scrotal temperature, defective spermatogenesis, decline in sperm concentration and motility and increased DNA damage of sperm. Mild to severe psychological stress and psycho pharmacological agents will decrease the testosterone and probably disrupt the spermatogenesis.

Table 3.2.7. Pollution and Radiation [4]

Air pollution	Reduces sperm motility.
Textile dye	Decrease the weight of the reproductive organ
Pulp/paper-mill	Reduces testis weight, count, motility and testosterone.
Ozone (Oxidant)	Reduce the sperm density by means of oxidative damage pathway.
Radio-frequency electromagnetic waves (RF-EMW)	Leads to oxidative stress in semen that negatively affects sperm and impairs fertility.
Chemotherapies and radiation	Utilized in the cancer treatment will severely affect sperm production

Diseases [4]

Mumps, sexually transmitted diseases, tuberculosis and febrile illness will cause temporary sperm decline.

Table 3.2.8.Antispermatogenic Plants [4, 55]

Azadirachta indica	Leaves are powerful spermicide
Carica papaya	Seeds affect cauda epididymis sperm motility, count and viability
Momordica charantia	Seeds have antisteroidogenic, antispermatogenicand androgenic properties
Embelia ribes	Spermicidal anti- androgenic activites.

Infections [3, 55, 56]

Antisperm antibodies presence is regarded as the indicator of chronic infections. *Chlamydia trachomatis* is the general cause of prostatitis and epididymitis. *E.coli* in the semen lowers the motilty of the sperm. *Candida albicans* exert its inhibition in sperm motility. *Ureaplasma urealyticum* have a negative effect on male fertility. *Mycoplasma hominis will* cause sperm tail abnormalities.

Table 3.2.9. Occupation and chemicals [4, 55, 57, 58]

Lead workers	Showed decreased spermatozoa count and decreased motility
Welders	Exposed to chromium and will have reduced sperm quality.
Exposure to copper	Associated to oligo/terato/ astheno- zoospermia
Professional drivers	Impairment of spermatogenesis
Pesticide/agricultural workers	1-2-dibromo-3-chloropropane and nematocide may affect spermatogenesis.
Formaldehyde	Leads to male sterility
Boric acid	Reproductive toxicant that reduces the testosterone
Aluminium	Reduces the weight of the reproductive organs and will impair fertility
Ammonium metavanadate	Toxic effect on reproduction

Table 3.2.10 Diet Factors [4, 55]

Gossypol	The toxic residue in cotton seeds inhibits sperm function and being examined as male birth control pill.
Estrogen and diethyl-stilbestrol	Broadly used in poultry, livestock and dairy industries.Increased exposure is responsible for prenatal testicular damage, post-natal testicular function depression and spermatogenesis.
Soyabeans-(isoflavone-phytoestrogens)	Long term use willl have adverse effect on the growth and function of male reproductive system will result in decrease count and fertility

Scrotal temperature [55]

Sperm needs temperature for about 3 to 4°C less than the normal temperature of the body for active production. This aspect is supported by the decrease sperm count in the pathologies like cryptorchidism, varicocele, patients constrained to wheel chair as in paralysis, drivers and in prolonged sauna exposure.

Table 3.2.11 Therapeutic drugs [4, 55]

Antineoplastic agents	Chlorambucil cyclophosphamide, busulphan and methotrexate
Drugs-Schizophrenia	Phenothiazines like thioridazine and chlorpromazine causes hypospermatogenesis, hyperprolactinaemia and impotence
Anti-bacterial drugs	Furacin and nitro furantoin significantly affect the spermatogenesis. Sulfasalazine causes oligozoospermia and reduced sperm motility Tetracycline derivatives will cause decrease sperm index Macrolide group – Neomycin, erythromycin and spiramycin affect fertility Penicillin group- Ampicillin, penicillin-G and dicloxacillin causes spermatogenic arrest. Aminoglycosides (gentamycin and neomycin) will alter the testicular functions..
Anti-malaria drugs	Quinine, chloroquine and quinacrine will inhibit leydig cell and steroidogenesis. Chloroquine will reduce the sperm motility. Pyrimethamine (prophylactic drug) causes spermatogenic arrest. Quinine causes morphological change in the testis and will suppress the spermatogenesis.

High blood pressure monitoring drugs	CCB (calcium channel blockers) and CNS depressant drugs will suppress the spermatogenesis
Drugs-Gastric problems	Will interfere in the production sperm and ejaculation
Chemotherapeutic agents	Will causes oligozoospermia and even will lead to azoospermia

Oxidative stress [59, 60, 61]

- Among various causes, oxidative stress (OS) has been attributed to have an effect on the fertility status and physiology of spermatozoa.
- Many pathological conditions such as cryptorchidism, infections in the male reproductive tract, varicocele, exposure to drugs, environmental factors, aging and smoking have oxidative stress as a common component.
- The time durability of spermatozoa in epididymis is longer in oligozoospermia which results in higher exposure to reactive oxygen species.

Pathogenesis [51,4,55,54,62]

- Condensation of sperm chromatin may be changed by exposure to the OP (organophosphorus) with higher susceptibility to denaturation of DNA and will affect the reproductive system adversely through protein phosphorylation mechanism.

- Klinefelter's syndrome (47 XXY) is the common chromosomal disorder that affects the growth of the testicle. Chromosomal abnormalities may interrupt cell division and the sperm production.

- Spermatogenic break-down will result due to Y chromosome (related to spermatogenesis) microdeletion. Genes required for the process of spermatogenesis are situated in azoospermia factor (AZF) region of Y chromosome. In azoosperimia deletion are related to the six azoospermia factor regions. In oligozoospermia deletions doesn't takes place inside AZF region and it takes place outside the region of deletion.

- Robertsonian translocations the major sex chromosomal aberration affects the structure and count in the semen and also several degrees of variation in the sperm. This take place when the two chromosome (acronetic) mingle together to form single

chromosome. This will result in the abnormal dicentric chromosome formation which will modulate the count, motility and morphology.

- Reciprocal translocations are a mutual exchange of information between the two chromosomes. This leads to the unbalanced sperm and their morphology. This results in severe oligozoospermia and azoosperimia in males.

- Mast cells release inflammatory mediators which directly inhibit sperm motility in potential reversible mode.

- Excess sugar in the blood will affect the quality of sperms directly and will gradually cause male infertility. In chronic diabetes, autonomous nervous system function gets damage which will result in the problems related to ejaculation and erection. It will directly effect fertility by causing sperm DNA damage

- Germ cell tumors will produce β-HCG (β–human chorionic gonadotropin) and AFP (α-fetoprotein). The increased β-HCG of the intra-testicular production of estradiol decreases/ inhibits spermatogenesis in contra-lateral testis and increased AFP will cause oligozoospermia

- Mumps, TB (tuberculosis) and STD (sexually transmitted diseases) may affect production of sperm through inflammation and male genital tract obstruction.

- *Chlamydial* infection may damage sperm parameters, acrosome reaction capacity and proportion of the DNA fragmentation, which will affect male fertility adversely.

- ASA (anti sperm antibodies) will impair fertility and quality of semen through impairing acrosomal reaction, the complement cascade will be invoked which will result in sperm lysis, motility inhibition, inhibits sperm penetration and capacitation. Sperm antibodies will impede the reproductive function and depends on where the antibodies are found as well as where the corresponding antigen located on sperm surface

- Lead and cadmium are considered to decrease the gonadotrophin binding which diminish the hormone secretion and will reduce the quality of the semen.

- Bisphenol A (BPA) the toxicant released in the environment in the period of industrialization affected the spermatogenesis by modifying the gene expression that is predient to the formation of sperm and also affects steroidogenesis by changing the effect of the epigenetic.

- Estrogen, its derivatives and diethylstilbestrol (synthetic analogs)) are responsible for depression of spermatogenesis. Exogenous estrogens have impact on fetal growth by inhibiting the sertoli cell development which will determine the life long capability for sperm production

- Smoking has negative correlation with cadmium in the blood and density of sperm. Smokers show the presence of elevated estradiol in serum, more leucocytes in semen, low sperm density, higher DNA fragmentation in sperm and decrease sperm penetration.

- Nicotine modifies the hypothalamic pituitary axis function which will affect the growth hormone, vasopressin, cortisol, oxytocin release then inhibits the luteinizing hormone and prolactin release which have negative impact on spermatogenesis.

- The testis is extremely susceptible to ethanol since it crosses the blood testis barrier and will depress the spermatogenesis

- Cocaine will induce injury in the testicle which could be associated to apoptosis and will involve the mitochondria-associated pathway or fasmediated pathway. Cocaine exposure will cuase the cytochrome release from mitochondria and following activation of caspase 9 and 3 in the testes and will play a important role in the cocaine induced germ cell loss or apoptosis in the testicle.

- There will be increase in the DFI (sperm DNA fragmentation index) with age

- In obesity SHBG (sex hormone binding globulin) will be decreased and free testosterone will be increased and results in the conversion of testosterone to estradiol in the adipose tissue. Decrease in the testosterone estradiol ratio will contribute to the spermatogenesis impairment.

- HPA (hypothalamus-pituitary-adrenal) axis controls spermatogenesis and reported to be involved in stress which raises the cortisol hormone levels and which will result in the involution of the testicle followed by fall in the testosterone. Stress is related to enhance ROS generation

Oxidative Stress as a common component of pathophysiological mechanisms [47, 59]

- Imbalance between the reactive oxygen species production and ability of the biological system to detoxify/repair the damage due to the reactive intermediates is known as oxidative stress.

- The production of reactive oxygen species by sperm is the normal physiological process. The imbalance between this ROS production and the scavenging activity is unfavorable to the sperm and is related with male infertility.

- The main source of production of ROS in semen is immature sperms and leukocytes. Spermatids and matured sperm are considered to be extremely sensitive to reactive oxygen species because sperm membranes are chiefly rich in the poly-unsaturated lipids.

- Inhibition in spermatogenesis will lead to abnormal sperm and give way to more ROS and which may overpower and reduce the antioxidant defense mechanism and end in oxidative stress

- Overload in free radical generation will frequently cause an error in the spermiogenesis which results in release of sperm from germinal epithelium with abnormal high level of the cytoplasmic retention:

- ROS alters the membrane integrity and will harm sperm morphology and motility and will lead in sperm death.

- The concentration of the marker lipid peroxidation MDA (malondialdehyde) was found to be twice as high in spermtozoa pellet suspension in asthenozoospermic and oligoasthenozoospermic males.

DIAGNOSIS

Fertility History [63]

- Infertility duration
- Childhood illness and problem in the development
- Diabetes,cancer, respiratory infections and previous surgery
- Sexually transmitted diseases
- Exposure to toxins, chemicals and radiation
- Medication history
- Family history related to reproductive problem

Developmental History [64]

- Cryptorchidism
- Orchiopexy (to treat cryptorchidism)
- Testicular trauma or torsion
- Pubertal development timing
- Mumps

Medical History [64]

- Diabetes
- Fever/viremia
- Prostatitis or Pyospermia
- Primary ciliary dyskinesia (immotile cilia syndrome)
- Chronic upper respiratory infections (affects sperm motility)
- Hormonal abnormalities-Thyroid disorder, elevated estrogen and hyperprolactinemia
- Urinary tract infections
- Sexually transmitted disease

Past Surgical History and Cancer Treatments [64]

Retroperitoneal and pelvic surgery will impair ejaculation. Testicular cancer may be present with male infertility before or after the treatment. 50 percentage of testicular cancer will affect sperm density before chemotherapy. Chemotherapy in lymphoma, sarcoma and leukemia will cause permanent sterility after treatment.

Physical examination [63, 64, 65]

Cremaster reflex

Intact reflex indicates integrity of sensory and the motor nerves

Inspecting the Pubis

Hair will be more/less abundant. Triangular shape pattern with no vertical extension will indicate hormonal disorder.

Inspecting the Penis

- Size, symmetry, color and hair distribution. Skin lesion, excoriations, warts, abrasions and tumors.
- **Phlebitis** - Tender/inflamed/nodular veins
- **Peyronie disease** - Penile curvature in erection
- Ulcer (Balanitis, granuloma inguinale, chancroid, herpes genitalis, primary syphlis or penile carcinoma).
- **Urethral meatus:** Erythema, discharge, vesicles, plaques, pustules and intraurethral warts.
- Urethritis or blockage in urethra (incontinence, dribbling) which will indicate urethral carcinoma or stricture.
- **Hypospadias** (incorrect position of urethral opening).

Inspecting the Scrotum

- Size and configuration
- Fungal or bacterial infections and skin lesion.
- Swollen area in the scrotum due to hernia in which peritoneum or portion of bowel will protrude into inguinal canal/scrotum which causes asymmetry.
- Asymmetrical swelling may also be a sign of a varicocele, tumour and hydrocele
- Scrotal thermography test to see the scrotal temperature

Palpating the Scrotal Contents

- Srotum (each half) should be checked for the presence of testicle (large ovoid mass)
- Epididymis (Ridge of tissue that lies vertically on postero-lateral surface of ovoid mass)
- Spermatic cord (Firm, non-tender column of the blood vessel and tissue ascends through and leaves the scrotal sac near to the groin).

Testicle

- Examine for cryptorchidism
- Examine for normal size - 2.5 to 5 cm, consistency, contour and tenderness.
- Nodular, very firm, or tender testis indicate cancer .
- Small and abnormal soft testis will indicate testicular atrophy or endocrine disorder
- Testicle could be measured with orchidometer. Detrioration in spermatogenesis frequently accompanied with small volume testicle. Normal volume is 20 ml.
- Smaller size and soft testis along with low sperm count strongly related with problem in sperm formation. Normal testis with low count may suggest probable obstruction.

Epididymis

- **Acute epididymitis:** Enlarged and tender epididymis when compared with the other side.
- **Epididymo orchitis:** Testis and epididymis could not be distinguished from each other in palpation. They are very tender and scrotum is generally inflamed.
- Chronic and painless induration of epididymis will indicate schistosomiasis (bilharzia), tuberculosis, or non-specific chronic epididymitis.
- Cystic mass near to the upper pole of testis which are separated from the testis and epididymis are generally spermatoceles that contain milky, thin fluid and sperm

Spermatic cord

- Swollen region in the spermatic cord will be cystic (hernia or hydrocele)
- Solid (rare connective tissue tumour or lipoma)
- Filariasis- Diffuse swelling/ induration
- Varicocele
- Absence of vas deferens or Tuberculosis

The rectal examination

- Warts, hemorrhoids, lesions, scars from trauma, mucous discharge and anal bleeding
- Tenderness in the prostate will indicate acute or chronic prostatitis.
- Prostate gland nodules (Cancer or BPH)

Laboratory Tests

Semen analysis [49]

Collection of semen for diagnostic or research purposes

- The sample is supposed to be taken through masturbation and should be ejaculated into a wide mouthed, clean, glass or plastic container (non-toxic for sperm).
- The container must be in ambient temperature of 20 °C - 37 °C
- The container must be placed on the bench or incubator (temp-37 °C) when the semen liquefies.
- Incomplete sample especially the first which is rich in sperm will be missing. And in that case second sample must be taken, again after 2-7 days abstinence period.

Liquefication

At room temperature the sample will normally liquefies within 15 mts, though rarely it will take up to 60 mts or more. Normally liquefied sample will contain jelly like granules (i.e- gelatinous bodies) which will not liquefy but these do not show any clinical significance. Presence of the mucus strands will interfere with the semen analysis.

Semen viscosity

High viscosity will interfere with sperm motility, concentration, biochemical markers and detection of antibody coated sperm.

Appearance of the ejaculate

A normal semen after liquification will have homogeneous, grey and opalescent in appearance. It will be less opaque when the concentration of sperm is very low and also the colour will be different. In haemospermia the colour will be red-brown; and yellow colour in jaundice or taking certain drugs and certain vitamins.

Semen volume

Semen volume is contributed principally by seminal vesicles and prostate gland and a small amount from bulbourethral glands/epididymides. Low volume is the feature of ejaculatory duct obstruction or congenital absence of vas deferens (bilateral), this is the condition in which seminal vesicles are developed poorly. Low volume may also be due to collection problem, retrograde ejaculation (partial) or androgen deficiency. High volume will be a sign of active exudation in active inflammation of accessory organs.

Semen pH

The pH exposes the balance between the values (pH) of different accessory gland secretions (alkaline seminal vesicular and acidic prostatic secretion). pH less below 7.0 with low semen volume and low sperm count will denote obstruction in the ejaculatory duct or congenital absence of vas deferens (bilateral), this is the condition in which seminal vesicles are developed poorly.

Aggregation of spermatozoa

The adherence of immotile sperms to each other or motile sperms to mucus strands or non sperm cell or debris

Agglutination of spermatozoa

Agglutination in particular refers to the motile sperms which stick to each other (head to head, tail to tail or mixed way). Agglutination implies the presence of anti sperm antibodies. Severe agglutination may affect the evaluation of sperm motility and sperm concentration.

Sperm motility

- **Progressive motility (PR):** Actively moving sperm, linearly or in large circle, and with regardless speed.
- **Non-progressive motility (NP):** Other type of motility with absence in progression, e.g. flagellar force that hardly displacing the sperm head, or once a flagellar beat will be observed and swims in small circles.
- **Immotility (IM):** No motility
- **Total motility:** Progressive motility (PR) + Non-progressive motility (NP)

Sperm vitality

It is clinically essential to identify whether immotile sperms are dead or alive. Vitality results must be evaluated with motility results. Presence of large percentage of vital but immotile sperm cells will indicate flagellum structure defect. High proportion of immotile / non viable sperm cells (necrozoospermia) will indicate epididymal pathology related sperm numbers.

Sperm concentration

- **Sperm concentration:** Number of sperms per unit volume of the semen
- **Total sperm number**: Total number of sperms in the entire ejaculation

The concept of normal spermatozoa

Sperm consist of head (with neck) and tail (with mid and principal piece). For a sperm to be referred normal, both the head and the tail should be normal.

Classification of abnormal sperm morphology

- **Head defects:** Large/small, pyriform, tapered, round, vacuolated, double heads, amorphous or any of this in combination.
- **Neck and midpiece defects:** Insertion (asymmetrical) of midpiece into head, abnormal thin thick or irregular, sharp bent or any of these combination.
- **Principal piece defects:** Mutiple, short, smooth hairpin bends, broken, sharply angulated bends, coiled , irregular width or any of this combinations.

Cellular elements other than spermatozoa

Epithelial cells from genito-urinary tract and leukocytes and immature germinative cells, and the latter two is together referred as round cells. Germ cells may comprise of round spermatids, spermatocytes, and rarely spermatogonia. Non sperm cells in ejaculation may indicate damage in the testicle (immature germ cells), efferent duct pathology (ciliary tufts) or accessory gland inflammation (leukocytes). Increased leukocytes (pyospermia, leukocytospermia) is related to infection and poor quality of sperm. Leukocytes may impair motility and DNA integrity by means of oxidative attack.

Test for antibody coating of spermatozoa [49, 63]

- Sperm antibodies may also be present without agglutination; likewise agglutination may be caused other than spermtozoa antibodies.
- Anti-sperm antibodies belong to two immunoglobulin - IgA and IgG.
- Blood test for antisperm antibodies may be carried out in man with reversed vasectomy and yet cannot impregnate the women or semen analysis showing clumping of sperms. ASA's will also be developed after injury to testis or genital infection. The two recognized test to assess the presence of antisperm antibodies are: The Immunobead test and Sperm Mar test.

Biochemical assays [49]

- **Secretory capacity of the prostate:** Zinc, acid phosphatase and citric acid
- **Secretory capacity of the seminal vesicles:** Fructose and prostaglandin
- **Secretory capacity of the epididymis:** L-Carnitine, neutral glucosidase and GPC
- Low fructose is the feature of ejaculatory duct obstruction, partial retrograde ejaculation, congenital absence of vas deferens (bilateral) and androgen deficiency.

Hormonal Levels [63, 64]

- FSH, LH, testosterone, PRL and estradiol (E2)
- Hormone test are specified particularly when sperm concentration is below 10 million per milliliter.
- Testosterone and FSH levels are generally assesed first. When level of testosterone is low then LH is measured.

Other sperm function tests [63]

- **Post-Ejaculatory Urine Sample:** Urine sample is asessed to identify sperm following the ejaculation which will indicate retrograde ejaculation and also can be used to test infections.
- **Postcoital Test (Cervical mucus penetration test):** To evaluate the effect of woman's cervical mucus to man's spermatozoa.

Ultrasound [63]

To find out the testis size or to detect tumors, cysts, tumors, varicocele or abnormal blood flow

Genetic Testing [63]

Tested in men with severe sperm deficient and with no obstruction.

Testicular biopsy [63, 64]

- To distinguish between maturation arrest and obstruction. When mature sperms are identified in biopsy it can be cryopreserved for IVF/ICSI cycle.
- Sertoli one syndrome (sperm producing cells in testis is found to be absent).

Fertilization Tests [63]

- **The Hamster Test (Micro-penetration assay test):** Sperm samples are used to fertilize the hamster eggs in which covering will be removed to allow sperm penetration. It is used to determine the best ART options for infertility men.
- **The Human Zona Penetration Test:** The test makes use of spermtozoa to fertilize the dead human eggs, usually taken from the ovary. Results will give the same suggestion like hamster test
- **Acrosome Reaction Test:** Induces the capability of the spermatozoa enzyme rich acrosome covering to dissolve

Investigative Tests [63]

Additional sophisticated lab test to measure the sperm function can also be carried out. They will assess the factors like level of cell damaging oxidants and computer aided sperm motility analysis.

Table 3.2.12 Differential Diagnosis [64]

Oligozoospermia Defect in sperm count	In count below 10 million /mL, FSH and testosterone should be assessed. In count below 5 million sperm per mL, karyotype or Y chromosome microdeletion test will be considered. Elevated FSH will signify primary testicular defect. Varicoceles are general cause of less sperm density.
Asthenozoospermia Defect in sperm motility	Varicocele and antisperm antibodies will be the cause for this defect.
Teratozoospermia Defect in sperm morphology	The major cases are idiopathic.Varicoceles and temperature influence to spermatogenesis is also potential cause**.**
Azoospermia Lack in sperm production	Non-obstructive azoospermia indicates lack in sperm production Obstructive azoospermia indicates failure in delivering the sperm to ejaculate, due to ductal obstruction. The information on size of testis and presence of vas deferens indicates the diagnosis. CBAVD (Congenital bilateral absence of the vas deferens) an obstructive azoospermia will be diagnosed through

	physical examination.
MultipleSemenAbnormalities Oligoasthenoteratozoospermia The defect in sperm count, motility and morphology	Most general cause is varicocele. Additional causes include environmental toxins, medications and cryptorchidism. In extreme cases (< 1 millionsperms/mL) there is increased occurrence of obstruction of male genital tract or genetic abnormalities.
Defects in Isolated Semen Parameters (Aspermia) No seminal fluids	May be due to retrograde ejaculation. Common causes include neurogenic abnormalities (spinal cord injury), multiple sclerosis, diabetes and use of α-blockers. Retroperitoneal surgery which includes the pelvic surgery and retroperitoneal lymph node dissection, may also cause impaired ejaculation.

TREATMENT

Antioxidants [4, 55, 66]

- Antioxidants like vitamin C and E, lycopene, β-carotene, zinc, folic acid, selenium, lactoferrin, papaya and lipophilic diet will improve sperm parameters.
- Carnitine (water-soluble antioxidant) obtained from human diet will protect DNA of sperm from free radical damage and apoptosis and will provide primary fuel for its motility.
- Polyunsaturated fatty acid, chiefly omega-3 fatty acids-docosahexanoic acid may be the most important sperm membrane fluidity determinant.
- CoQ10 and vitamin E were observed to be effective in Oligozoospermia.

Hormone therapy [63]

- In hypogonadism and gonadotropin deficiency GnRH (Gonadotropin-releasing hormone) will be useful. GnRH may also be helpful in restoring the sperm production following to chemotherapy treatment.
- Sperm production rarely respond to the low doses of testosterone; estrogen and testosterone; clomiphene citrate (Clomid); menotropins (Repronal, Pergonal), human follicle-stimulating hormone (Gonal-F, r-hFSH) and human chorionic gonadotropin (hCG).
- An enzyme, aromatase inhibitors block aromatase, is the main source of estrogen which present in various main body tissues. They are letrozole. (Femara) and anastrozole (Arimidex) whih will be helpful in male infertility related to unusual testosterone-to-estrogen ratios.

Nonhormonal Agents [63]

- Bromocriptine – Parlodel is given in male infertility related with excess prolactin harmone.
- Infections that affect male fertility will be treated with the antibiotics.
- Men with less count will be treated with antihistamines which will block mast cells.

Treatment for Antisperm antibodies [63]

- IUI (intrauterine inseminations) to avoid the cervical mucus or IVF (In Vitro Fertilization) in any case of antibody type.
- Antisperm antibodies can be treated with steroids.

Surgical Procedures [63]

- Ejaculatory duct obstruction may be treated by scraping or excising the part where prostate gland enclose the urethra and through reconstructing the duct.
- Undescended testicles (in young boy) will be repositioned through surgery to prevent later on infertility.

Table 3.2.13: Assisted Reproductive Technologies [67, 68]

Artificial/Assisted Insemination	Sperm by concentrating before insemination or by sperm donation is introduced in to the uterus. Used to treat male infertility with weak sperm, low count or total testicular failure to produce sperm
In vitro fertilization (IVF)	Fertilization takes place outside the body. Used in when fallopian tubes of the women is blocked or man producing too little sperms.Women are treated with the drugs that will cause to produce multiple eggs from the ovaries and once matured, eggs will be removed. Eggs are put in to the dish in a lab along sperm for fertilization. Later than 3-5 days, healthy embryos will be implanted in to the uterus.
Zygote intrafallopian transfer (ZIFT) or Tubal Embryo	Transfer is alike to IVF. Fertilization takes place in the lab. Very young embryo will be transferred in to the fallopian tube.
Gamete intrafallopian transfer (GIFT)	Transferring of sperm and eggs in to the fallopian tube. Fertilization takes place inside the woman's body.
Intracytoplasmic sperm injection (ICSI)	Used in men with serious sperm problems or older couples or in failed IVF efforts.A single sperm will be injected in to the matured egg. After that the embryo will be transferred to uterus/fallopian tube
Donation of Gamete and Embryo	In assisted insemination or IVF or its variants, the issue of egg, sperm or embryo donation becomes applicable. Sperm, eggs and embryos may be frozened through cryopreservation and these will be thawed later and will be offered for use to anybody who needs them, predominantly women or men with diminished egg/sperm.

Lifestyle Changes [63]

- Smoking should be avoided
- Overweight men can make an effort to reduce weight
- Should take adequate rest; regular and moderate exercise
- Stress reduction techniques will develop fertility
- Tight underwear cause no hazard to fertility, however there will be no harm in looser clothing
- Avoid hot showers and steam rooms to prevent over heating to testis

Preventive measures [3]

- Sex education
- Public health and hygiene
- Control of STD (sexually transmitted diseases)
- Rectification of nutritional deficiencies
- Early on treatment for abnormal conditions
- Prevention of the damage from chemical, trauma,.heat and x-ray exposure
- Cotton seed oil, hydrogenated oils, saturated fats, trans-fatty acids, palm and coconut oil should be avoided.

3.3. DRUG REVIEW

Table 3.3.1: Traditional uses of the constituents of CKC as referred in Siddha literature

Botanical & Mineral name	Parts	Action & Uses
Tribulus terrestris	Fruit	Thathu sheena rothi[69], Spermatorhoea[17] Aphrodisiac[70], Testosterone booster[71] Impotency, Increases the semen[72]
Curculigo orchioides	Rhizome	Thatu pushti[69], Bogum kuriaivu[73] Aphrodisiac [69, 40, 74] Sexual debility [70] Impotency [75]
Mucuna prurita	Seed	Nocturnal emission [69] Thathu viruthi [69, 75] Male virility, Spermatorrhoea, Male sexual Dysfunction, Hyperprolactinemia[70] Aphrodisiac [40, 74]
Madhuca longifolia	Flower	Aphrodisiac [69]
Cinnamomum tamala	Leaf	Thathunashtam [69, 40, 74]

Cinnamomum verum	Stem bark	Thatunashtam [69, 40, 74] Aphrodisiac [40] Aanmai peruki[76]
Syzygium aromaticum	Flower bud	Shena vinthu [69] Sukilanashtam [40, 74] Aphrodisiac [72]
Maerua arenaria	Root tuber	Thathu viruthi [69]
Coscinium fenestratum	Stem bark	Tonic effects [40] Astringent [69]
Moringa oleifera	Seed	Neerthu pona vinthu [40] Veneral disease [77] Premature ejaculation[69] Vinthu thadipu [69 74] Aphrodisiac [72]
Mesua ferrea	Flower	Vinthu nashtam[74] Aphrodisiac [75] Male gonorrhoea [69] Impotency[71]
Lawsonia inermis	Seed	Vaginal discharge, Menorrhgia, Leucorrhoea [78]
Bombax ceiba	Gum	Sukilam balapadum [40] Spermatorrhoea [77] Aphrodisiac [69] Vinthu undakum[79]
Vitis vinifera	Dried fruit	Semen in suspension[17] Immunostimulant,Anti oxidant, Aphrodisiac [80] Enriches blood [79] Anmai kuraivu [73] Sukila viruthi [69]
Bambusa aurundinaceae	Salt	Aphrodisiac [69 40 77]
Ilicium verum	Fruits	Anmai kuraivu [73] Thathu viruthi [74]
Phoenix dactilifera	Unripe fruit	Inferility [80] Aphrodisiac [69 40 80] Impotence, Diabetes [69]
Cyperus rotundus	Rhizome	Aphrodisiac [71 72] Thathu viruthi [69]
Costus speciosus	Root	Thatunashtam [40 74] Veeriya nashtam [69] Aphrodisiac [69 71 72 80]
Cuminum cyminum	Fruit	Cooling effect [72] Aphrodisiac [80]
Myristica fragrans	Seed	Vinthu nashtam [69] Vinthu kuraivu, Aphrodisiac [69 40 76], Spermatorrhoea [77] Thatu nashtam [69 74] Impotency [72]
Glycyrrhiza glabra	Root	Thathu sheena rothi [69] Thatunattam [69 40 74] Aphrodisiac [79]
Asphaltum punjabinum	Parpam	Inthriya nashtam [74] Spermatorrhoea [81] Infertility, Bioenhancer, Aphrodisiac [82]

Research studies done on the ingredients of CKC

1. ***Tribulus terrestris* Linn (Zygophyllaceae)**

Synonyms: Tamil- Nerunjil; Sanskrit-Trikanta,Gokshura; Eng- Puncture Vine [70]

Habit and Habitat: Annual small herb which grows to the height of 10-60cm. It has carpel fruits with stellate appearance.[83]

Traditional uses: Male reproductive disorders[84] premature ejaculation, spermatorrhoea[85] Infertility [86] oligospermia (thathu sheena rothi), testosterone booster, increases the semen, impotence and aphrodisiac action [Table 3.3.1].

Chemical Constituents: There are three groups of active phytochemicals in Tribulus terrestris - 1) Dioscin: Increases sexual energy and free testosterone level 2) Sterols: Facilitates meiosis process during the spermatogenesis. Protects the prostate from swelling 3) Steroidal saponins: Effects on immune system .[87, 88]

Research works

- Reported to be effective in treating anti-sperm antibodies. [88]
- Clinical studies for 3 months with the treatment of *Tribulus terrestris* had showed improvement in sperm count and motility and in erectile dysfunction (ED). [8]
- The active chemical PTN (protodioscin) a steroidal glycoside given in treating hypogonadism patients for 30 to 90 days had enhanced the testosterone and LH level. [89]
- Exposure to Cd (cadmium) will have an effect on hypothalamus-pituitary-testicular axis, and will decrease FSH, LH and testosterone. *Tribulus terrestris* due to the antioxidant effect and metal chelating effect had protective effect on testis against cadmium induced testicular damage in experimental rats.[84]
- *Extraxt of the Tribulus terrestris* promotes the production of testosterone and will build up the muscle mass and helps in the growth of male characters. Increases the production of red cells and the blood circulation. [90]
- Rabbits that received protodioscin had proerectile activity. [83]
- *Tribulus terrestris* reported to have aphrodisiac activity in sexually sluggish aged males. [91]
- *Tribulus terrestris* for eight weeks in Sprague dawley rats had increased ICP (intracavernous pressures) and sexual activity. Furostanol (glycoside fraction) of

Tribulus terrestris at 5, 10, 25 mg/kg dose in castrated rats for 14 days increased the sexual stimulation. [92]

- Lyphophilized powder (100 mg/kg) in male albino rats had showed anabolic effect and showed increase in the body and reproductive organs weight without any toxicity. [93]

2. *Curculigo orchioides Gaertn* (Amaryllidecae)

Synonyms: Tamil- Nilapanai kizhangu; Sanskrit-Taalmuuli; Eng- Golden eye grass [70]

Habit and Habitat: Small herb with short, stout and elongated tuberous root with 10 cm long. Distributed in Nepal, India, China, Australia, Japan and Malaysia. [94]

Traditional uses: Venereal diseases [95] male infertility, spermatorrhoea [96] sexual debility (bogum kuriaivu), seminal strenght (thatu pushti), impotency and aphrodisiac action [Table 3.3.1]. Even wild beer eat few days before sexual intercourse [75]

Chemical Constituents: Glucosides: curculigoside - A, B, C and D, Cycloartane – saponins: curculigosaponin- G and I, Phenolic glucosides: orchiosides - A and B, Phenolic glycoside orcinosides - A, B and C [97]

Research works

- .Ethanolic extract of *Curculigo orchioides* (100 mg/Kg b.wt) in rats showed increased number of spermatocytes and spermatids. [98]
- The lyophilized aqueous extract of *Curculigo orchioides* (200 mg/kg b.wt) had showed progression in sexual activity. [99]
- The aqueous extract of *Curculigo orchioides* ameliorates sexual dysfunction in rats against the streptozotocin induced hyperglycemic stress. [100]
- The aqueous extract of *Curculigo orchioides* ameliorates decreased spermatogeneis and heat shocked protein formation in rats and had protected reproductive organs from the heat induced sexual dysfunction. [94]
- *Curculigo orchioides* relaxes corpora cavernosa smooth muscles in order that more blood will be pumped into and will help to overcome the erectile impotence. [96]
- Ethanolic extract of *Curculigo orchioides* comprises of androgen and adaptive effect.[96]
- Antioxidant and immunostimulant activities are reported. [98]

3. ***Mucuna prurita*** **hook (Leguminoceae)**

Synonyms: Tamil- Poonaikkaali; Sanskrit-Kanduraa, Adhigandhaa**;** Eng- Cowhage [70]

Habits and habitat: Climbing, annual shrub by means of long vines which reach above 15 m in length, seen in India, Caribbean and Africa. Contact with seed pod and young foliage produce severe itchiness.[101]

Traditional uses: Impotency, male reproductive disorders, [102] in hyperprolactinemia, [101] male fertility and vitality [103,104] male virility, spermatorrhoea, sexual dysfunction, increase the sperm (thathu viruthi), nocturnal emission and aphrodisiac action [Table 3.3.1].

Chemical Constituent: Mucunine mucunadine, mucunadinine and prurienidine. Copper, manganese, magnesium, iron and zinc.[105]

Research works:

- Seed powder and the extract of *Mucuna pruriens* reported to have fighting effect against the stress mediated compromise in spermatogenesis.[106]
- *Mucuna pruriens* have positive effect in improving spermatogenic loss and provide it as treatment of choice for extracting quality sperm to be used in IVF procedures.[106]
- Improves sperm formation by restoration of endocrine axis and the testicular homeostasis which leads to improve the semen quality. [106]
- Reported to have anti-diabetic, anti oxidant and adaptogenic activity. [106]
- Treatment with *Mucuna pruriens* in male infertile groups showed increase in sperm count and motility. [107]
- Ethanolic extract of *Mucuna pruriens* (dose of 150, 200, 250 mg/kg p.o) reported significant increase in sexual activity in wistar rats. [108]
- Improves the antioxidant defence system and protects the sperm damage in ageing.[75]
- *Mucuna pruriens* corrects the fructose content in semen of the infertile males. [75]

4. ***Madhuca longifolia*** **Linn (Sapotaceae)**

Synonyms: Tamil- Iluppai; Sanskrit- Gudapushpa, Madhusrav; Eng- South Indian Mahua [70]

Habits and Habitat: Large evergreen tree. Grown in India, Srilanka and Nepal.[109] Creamy white flowers consist of many stamens, with hairy ovary and long style.[110]

Traditional uses: Blood purifier**,** given in low count [111] to increase seminal fluids [109] aphrodisiac action [Table 3.3.1]. Flowers which are dried is used in alcohol production which will preserve the drugs. [112]

Chemical Constituents: Tannins, flavones, mallotus-AB, chalcones, srottlerin, isorottlerin, new oleanene,cardenolide, gum, volatile oil, saponins, triterpenoid and alkaloids.[113] Consists of iron, arginine, calcium, phosphorus, potassium, sodium and magnesium.[114] Facilitate the extraction of the active components from herbs and helps in the absorbation of active components from gastrointestinal tract. [112]

Research works

Antioxidant activity [113] and anti- bacterial action [114]

5. *Cinnamomum tamala* Nees & Eberm (Lauraceae)

Synonyms: Tamil- Lavangappattiri, Sanskrit- Tamaalpatra, Tejapatra, Eng-Indian Cassia.[70]

Habit and Habitat: Tree is generally distributed in South America, Asia, India, Australia and Pacific region. Single tree normaly produces 10 to 25 kg dry leaves.[115]

Traditional uses: Astringent, stimulant, diabetes and cardiac disorder[115] low vitality [116] Oligozoospermia (thathunashtam) [Table 3.3.1].

Chemical Constituents: Eugenol (chief constituent), cinnamaldehyde, spathulenol, methyleugenol, viridiflorene and aromadenendrene. [115]

Research work

Anti-hyperglycemic[117] immuno-modulatory [118] antioxidant and anti hyperlipidemic activities.[115]

6. *Cinnamomum verum* Persl (Lauraceae)

Synonyms: Tamil- Elavangappattai; Sanskrit- Daarusitaa, Varaanga; Eng- Cinnamon and Ceylon Cinnamon.[70]

Habit and Habitat: Native to Srilanka and South India. Cinnamon is the dried out inner-bark of the tree. [119]

Traditional uses: Aphrodisiac (aanmai peruki), Oligozoospermia (thatunashtam) [Table 3.3.1].

Chemical Constituents: Cinnamaldehyde and eugenol. [120]

Research work

- Cinnamon bark extract demonstrated significant increase in the sperm count and motility and weight of the reproductive organ. [121]
- Antioxidant activity [119]

7. ***Syzygium aromaticum*** **(Linn.), Eberm (Myrtaceae)**

Synonyms: Tamil- Lavangam, Kiraambu; Sanskrit- Lavanga, Eng- Clove [70]

Habit and Habitat: Evergreen tree grows up to the height of 10 to 20 m. Native to Indonesia, India, Mauritius, Zanzibar and Ceylon. [122]

Traditional uses: Oligozoospermia (shena vinthu, sukilanashtam), aphrodisiac action [Table 3.3.1] prevent premature ejaculation.

Chemical constituents: Eugenol, Eugenin, eugenol acetate, caryophyllene, triterpene, apigenin, tannins, quercetin, benzaldehyde and kaempferol. Minerals present are selenium, iron, manganes, potassium and magnesium. [123,124]

Research work

- Hexane extract (15 mg/kg p.o) of the flower buds at low dose increased the testosterone level. [125]
- Hydro alcoholic extract (50%) of the clove in mice demonstrated aphrodisiac activity. [124]

8. ***Maerua arenaria*** **Hook. f. & Th. (Capparidaceae)**

Synonyms: Tamil- Bhumichakkarai; Sanskrit- Madhusravaa, Piluparni; Eng- Earth sugar root. [70]

Habit and Habitat: Woody twine straggler grows in the South Indian dry forest. Outer stele region of the roots are known to show peripheral vascular bundles. [126]

Traditional uses: Sterility[127] increases sperm (thathu viruthi) [Table 3.3.1] and aphrodisiac action. [128]

Chemical Constituents: Phytosterols, saponins, alkaloids, amino acids, carbohydrates, glycosides [126] and lupine triterpenoid – [12]

Research work:

Antipyretic activity [130] and aqueous extract (800mg/kg p.o) of *Maerua arenaria* exhibited significant reduction in glucose level. [131]

9. ***Coscinium fenestratum*** **Gaertn (*Menispermaceae*)**

Synonyms: Tamil- Maramanjal; Sanskrit- Harichandana; Eng- False Calumba[70]

Habit and Habitat: Tree Turmeric is broadly grown in Africa, Asia, Indochina, India and Sri Lanka [132]

Traditional uses: Tonic, astringent [Table 3.3.1] diabetes mellitus[133] and anemia [134]

Chemical Constituents: Berberine (major active component) [66] sitosterol, ceryl alcohol, saponin, hentriacontane, oleic acid and palmitic acid [133]

Research work

Alcoholic extract exhibits considerable increase in the hepatic antioxidant enzymes that protects the cells against the free radical damage and in normal and diabetic rats showed significant decrease in blood glucose level.[132]

10. ***Moringa oleifera Lam*** **(Moringaceae)**

Synonyms: Tamil- Murungai; Sanskrit- Shigru; Eng- Drumstick, Horseradish[70]

Habit and Habitat: Perennial, short and slender tree which grows to the height of 10 m.Broadly distributed in sub-himalayan ranges, Africa, Arabia, Sri Lanka and Madagascar. Brown, triangular and pendulous pods splits in length-wise to three parts when it is dried. Contains twenty seeds which are dark brown and contains three papery wings. [135]

Traditional uses: Venereal disease, low viscous semen (neerthu pona vinthu), high viscous semen (vinthu thadipu) aphrodisiac, premature ejaculation [Table 3.3.1].

Chemical Constituents: Methionine and cysteine and methionine (similar to human and cow's milk and eggs of chicken) [136]. Oil from the seeds is commercially familiar as ben oil, related to olive oil which contains stearic, palmetic, oleic acids and behmic [135] Moringinine and the alkaloids demonstrates the effect of the seeds. [137]

Research work

- Aqueous extract of *Moringa oleifera* (100, 200 and 500 mg/kg) improve sexual behaviour in rats (male) and increases the sperm count [138].
- Antitumour activity [136] and anti-pyretic activity [137]

11. ***Mesua ferrea*** **Linn (Guttifereae)**

- **Synonyms:** Tamil- Sirunagappo; Sanskrit- Naagakeshara, Naagapushpa, Eng- Iron wood. [70]

Habit and Habitat: Indigenous to SriLanka and also found to be cultivated in Malaya, India, Peninsula, southern Nepal and Indochina [139]. Flowers consist of white petals and has a center with many yellow clour stamens.[140]

Traditional uses: Oligozoospermia (vinthu nashtam), impotency, male gonorrhoea and aphrodisiac action [Table 3.3.1].

Chemical Constituents: Mesugin, Mesuferron, Mammeisin, Mesuanic acid, and Sitosterol. [141]

Research work

Anti-cancer activity [142] and ethanolic extract demonstrated antioxidant activity [139]

12. Lawsonia inermis Linn (Lythraceae)

Synonyms: Tamil- Maruthani, Sanskrit- Madayanti; Eng-Henna [70]

Habit and Habitat: Herbaceous and biennial shrub with greyish brown bark colour. Native to North Africa and south west Asia. Older trees have spine tip. Many, smooth and pyramidal seeds are.present. Brown seed coat which is thick and hard.[143]

Traditional uses: Henna signifies fertility. Seeds are given in liver disorder and dysentery [143] vaginal discharge, menorrhagia and leucorrhoea [Table 3.3.1].

Chemical Constituents: Proteins, carbohydrates, fibers, fatty oils- behenic acid, stearic acid, oleic acid, palmitic acid, arachidic acid and linoleic acid. [143]

Research work

- Ethanol extract of the powdered seeds failed to prove antifertility activity [143]
- CNS depressant activity [144]

13. Bombax ceiba Linn (Bombaceae)

Synonyms: Tamil- Ielavampisin; Sanskrit- Mocharas, Eng- white silk cotton tree [70]

Habit and Habitat: Broadly spread in Indian forest to the height of nearly 1500 m.Light brown gum is like the galls and will slowly change in to opaque dark brown colour. [145]

Traditional uses: Known to be called as sukila bandhini since it keeps semen in suspension state.[2] Gives strength to semen (sukilam balapadum), spermatorrhoea, increases sperm (vinthu undakum), aphrodisiac [Table 3.3.1] impotency, sterility, and nocturnal emission [146]

Chemical Constituents: Tannic acid, gallic, D-galacturonic acid, D-glactose, aldobiuronic acid, L-arabinose[145] naphthoquinones, naphthol, anthocyanins, polysaccharides, lupeol and shamimin [146]

Research work

Anti cancer, aphrodisiac, antioxidant, anti-HIV, antimicrobial activity and hypoglycemic [146]

14. Vitis vinifera Linn Eberm (Vitaceae)

Synonyms: Tamil- Draksha; Sanskrit- Draakshaa, Kishmish; Eng- Wine Grape [70]

Habit and Habitat: Native to southern Europe and western Asia and cultivated all over India. [147, 148]

Traditional uses: Increases sperm (sukila viruthi), sexual debility (anmai kuraivu), enriches blood, aphrodisiac, immunostimulant [Table 3.3.1] stress, antidiabetes and cardiovascular diseases [149]

Chemical Constituents: Flavonoids, polyphenols, procyanidins, resveratrol, anthocyanins, proanthocyanidins. [149]

Research work:

Anti-microbial activity, Antioxidant [149] adaptogenic and immunomodulatory activity [148]

15. Bambusa aurundinaceae willd (Poaceae)

Synonyms: Tamil- Bamboo-manna, Moongiluppu, Sanskrit- Vanshalochana, Tvakkshiri; Eng- Spiny or Thorny Bamboo [70]

Habit and Habitat: Spread in India excluding Himalaya and indo-gangetic plain. The internal stalk of the female plant consists of silicious- bluish white colour concretion called as tabashir [150]. Medicinal salt [17]

Traditional uses: Aphrodisiac, stimulant and cooling effect [17]

Chemical Constituents: Ninety percentage of silica, peroxide of iron, potash, alumina, urease, lime, cholin, vegetable matter and cyanogenetic glycoside [150]

16. Ilicium verum Hook.f. (*Magnoliaceae*)

Synonyms: Tamil- Anasippo, Takkola; Eng- Star Anise [70]

Habit and Habitat: Tree (medium size) which give aggregate and capsule fruit. Fruits are star shaped and radiating to 5-10 boat formed sections with shiny brown

seeds which has high oil content. Before ripening fruits should be picked and should be dried. Spreaded in Asia, China and native to Vietnam.[151]

Traditional uses: Increases sperm, thathu viruthi and impotency (aanmai kuraivu) [Table 3.3.1].

Chemical Constituents: Anethole (major active compound), tannins, limone, α-pinene, β-phellandrene, farnesol, α-terpineol and safrol [151]

Research work

Antioxidant effect [151] and androgenic effect [152]

17. ***Phoenix dactilifera*** **Linn (Arecaceae)**

Synonyms: Tamil- Perichchankay; Sanskrit- Kharjuuraka; Eng- Date palm [70]

Habit and Habitat: Main food in North and the Middle-East Africa, native to the Gulf countries.[153] One-seeded berry fruits with epicarp that enclose the fleshy-mesocarp. Seed is enclosed by the hard-endocarp. [154]

Traditional uses: Infertility, impotence, diabetes and aphrodisiac action [Table 3.3.1]. Act against alcohol toxicity [153]

Chemical Constituents: Iron, zinc, selenium, copper, calcium, cobalt, magnesium, fluorine, manganese, phosphorus, potassium, sodium, boron and sulfur.[155] Flavonoid and the estradiols that has positive effect on sperm quality. [156]

Research work

- Activate cellular immune system in the mice [157]
- Aqueous and the methanolic extract showed stimulatory effect on the tissue of the bone marrow for haemopoietic activities [158]
- Aqueous extracts showed antioxidant activity [159]
- Aqueous extract of the dates ameliorates atrazine testiculo-toxicity effect and improved the sperm parameters and the testicular oxidative enzymes. [153]

18. Cyperus rotundus **Linn (*Cyperaceae*)**

Synonyms: Tamil- Koraikkizhangu; Sanskrit- Mustaka; Eng- Nut Grass [70]

Habit and Habitat: Common perennial herb similar to grass normally grows to 7 to 40 cm in tall. Rhizomes at first are white, fleshy with scaly leaves and later turn into dark brown, wiry and fibrous. Indigenous to India. [160]

Traditional uses: Spermatorrhoea[161] increases sperm (thathu viruthi) and aphrodisiac action [Table 3.3.1].

Chemical Constituents: Flavonoids, polyphenols, ascorbic acid, sesquiterpene, cyperenone, cyperene, cyperol, mustakone, copadiene, rhamnopyranoside and kobusone [162]

Research work:

- Aqueous extract exhibited maximum preventive effect against testicular damage (400mg/kg).[16]
- Antioxidant, antimicrobial and antidiabetic activity [162]

19. Costus speciosus (Koen.) Sm. (Costaceae)

Synonyms: Tamil- Ven Kottam, Sanskrit- Kembuka, Kebuka, Eng- wild Ginger [70]

Habit and Habitat: Erect plant which grows to the height of 2.7 m and has tuberous root stock. Indigenous to Malay Peninsula of the Southeast-Asia. [164]

Traditional uses: Given in aphrodisiac, thatunashtam, veeriya nashtam (Oligospermia) [Table 3.3.1] spermatorrhoea[165]

Chemical Constituents: Costunolide, flavonoids, eremanthin, phenol, ascorbic acid, β-carotene, glutathione and α-tecopherol. Diosegenin the precursor in steroidal hormone synthesis and hence attained value in drug industry. Costunolide and eremanthin [166,167,168]

Research work

- Normal glycemic effect in streptozotocin-stimulated diabetic rats [168]
- Antistress activity [164]

20. Cuminum cyminum Linn (Apiaceae)

Synonyms: Tamil- Cheerakam; Sanskrit- Ajaaji; Eng- Cumin [70]

Habit and Habitat: Small, tender, annual plant, grows to the height up to 30-cm. Originated in Mediterranean region, Egypt, Syria and grows broadly in Iran and also in Turkey. Commonly mentioned in bible, mostly by Isaiah and Mathew. Yellow-brownish grey fruit (cumin seed) and contains nine protuberances. [169]

Traditional uses: Infertility, testicle swelling [134] sexual stimulant [169] aphrodisiac and cooling effect [Table 3.3.1].

Chemical Constituents: It consists of cuminol, cymene, carvone, terpene and cuminaldehyde. [170]

Research work

- *Cuminum cyminum* (0.25 g/kg p.o) to alloxan induced diabetic-rats for 6 weeks exhibited decrease in blood-glucose level and increase in the haemoglobin level.[171]
- Antistress and Antioxidant activity. [172]

21. Myristica fragrans Houtt (Myristicaceae)

Synonyms: Tamil- Jaathikkai; Sanskrit- Jaatishasya, Jaatiphala; Eng- Mace, Nutmeg [70]

Habit and Habitat: Evergreen bushy tree which grows to the height of 10–20 m. Distributed in Indonesia, India and Srilanka. Dried out kernel of the broad-ovoid shaped seeds. [122]

Traditional uses: Spermatorrhoea, Oligozoospermia (thatu nashtam, vinthu kuraivu, vinthu nasham), impotency, aphrodisiac [Table 3.3.1] and raises the blood circulation. [122]

Chemical Constituents: Myristicin, lignan and eugenol (demonstraed to maintain the antioxidant level). [173]

Research work:

- Ethanolic extracts (50%) of nutmeg in mice improved the sexual activity in mice significantly. [122]
- Seed extract decreases the deleterious radiation effect on testis. [173]

22. Glycyrrhiza glabra Linn (Leguminoceae)

Synonyms: Tamil- Athimathuram; Sanskrit- Madhurasaa, Atirasaa; Eng- Liquorice[70]

Habit and Habitat: Indigenous to Asia and the Mediterranean region. Consist of fibrous and soft, bright- yellow tap root. [174]

Traditional uses: Sexual debility[175]enlarged prostate[176]erectile dysfunction [177] male infertility[178] anemia (a plastic) [179]spermatorrhoea [134] Oligozoospermia (thathu sheena rothi, thatunashtam) and aphrodisiac [Table 3.3.1].

Chemical Constituents: Glycyrrhizin, glabrolide, glabrin A&B, isoglabrolide, glycyrrhizinic acid, glycyrrhetol, triterpene sterols, triterpenoids, saponin, coumarins and isoflavones.[180]

Research works

- Aqueous root extract demonstrated immunomodulatory activity. [179]

- Extract showed radio-protective activity, antidepressant effect [181] and antioxidant activity. [182]
- Inhibits the growth and also spread of the androgen-refractory cancer of prostate. [182]
- The extract showed aphrodisiac activity (150, 300 mg/kg p.o -28 days) in rats. [93]

23. ***Asphaltum punjabinum***

Synonyms: Tamil- Kalmatam, Uerangyum; Sanskrit- Shilajit, Silaras; Eng- Bitumen, Mineral pitch [70]

Habit and habitat: Himalayan rock exudation in India, also in Nepal, Bhutan, Tibet and China. Gomutra Shilajit (Iron-shilajith) blackish-brown in colour is qualified as the best. [183]

Traditional uses: Natural mineral rasayana and enhances the bioavailability of the herbs in the body. [183] Have effect on reproductive and the nervous tissues. Has specific action on endocrine system. Given in treating infertility [184] thyroid disease [185] Oligozoospermia (inthriya nashtam), sexual weakness, spermatorrhoea [Table 3.3.1] genitourinary diseases and anaemia.[186]

Chemical constituents: Shilajit-humus consists of organic- 60 to 80%; mineral-20 to 40%; trace element- 5% (Fe, Zn,Ca, Mg, Cu, Mn, P, Mo).[187,188,] hippuric acids, gums, traces of resin, fatty acids and albuminoids.[184] Proceed as synergistic enhancer when added with other drugs. Organic substance transports minerals to their target cell. [189] Urinous odour is due to the presnce of benzoates and benzoic acid (main active principles) and activity of shilajit is due to the presence of fulvic acid and humic acid (primary active components).[190] Fulvic acid (carrier molecule) transports nutrients in-to deeper tissues and also removes toxins. Fulvic acid facilitate the absorption of iron and helps in its bioavailability to bone marrow-stem cells to produce blood. [185]

Research works

- Spermatogenic activity is proved in rats and serum testosterone was higher in male rats treated with Shilajit. [11]
- The semen quality is enhanced due to the inclusion of Shilajit (processed) components into semen of the oligozoospermic patients. [11]
- Immunomodulator activity [189] and antioxidant activity. [186]

24. Adhatoda vasica Nees (*Acanthaceae*)

Synonyms: Tamil- Aadaathodai; Sanskrit- Vaasaka; Eng- Malabar nut [70]

Habit and Habitat: Herbaceous bush (sub), indigenous to India and grows in the open plains, generally in lower Himalayan ranges. Grows up to 1300 m beyond the sea level. Distributed in Punjab, Assam, Bengal, Ceylon, Singapore and Malaysia. Small capsule fruits with four seeds. [191,192]

Traditional uses: Fruits posess the main potential of the herb. [192]

Chemical constituents: Alkaloids (in defatted seeds) – vasicinol, vasicine and vasicinone [134]

Research works

Ethanolic extract of the leaf (800 mg/kg p.o) for 15 days showed significant radio protective effect on radiation induced-chromosomal damaged cells of bone marrow in rats. [193]

25. Alternanthera sessilis Linn (Amaranthaceae)

Synonyms: Tamil- Ponnonkanni keerai; Sanskrit- Matsyaakshi; Eng- Sessile joy weed [70]

Habit and Habitat: America, India and Africa. Seed is ovate, orbicular, inverted and compressed. [194]

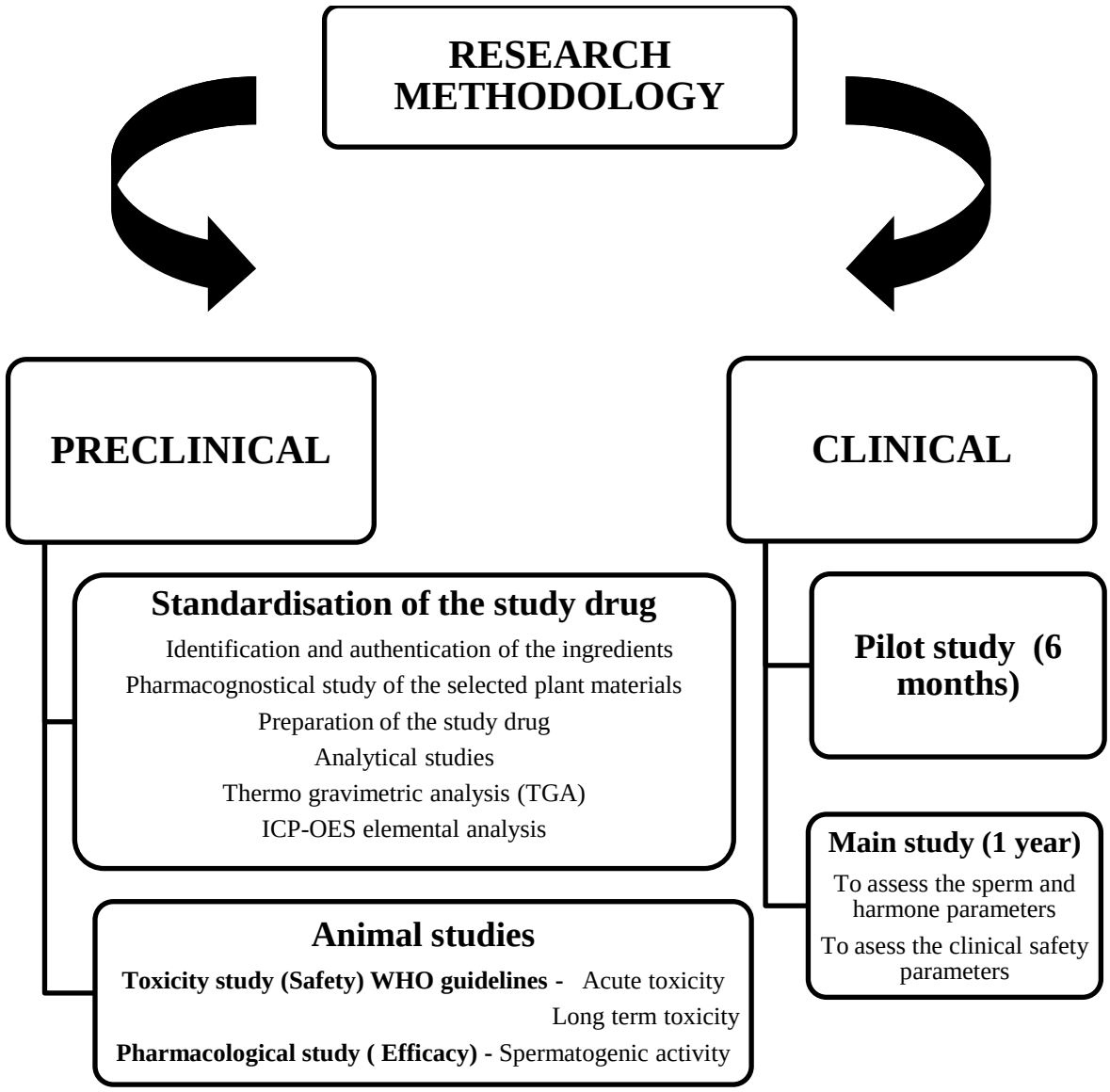
Traditional uses: Shoot with added drugs restore virility.[98] Roots treat spermatorrhoea. [195]

Biochemical constituents: Seed oil – Myristic, ricinoleic, oleic, palmitic, linoleic acid and stearic. [196]

Research work

The genus claim immunomodulator effect [19]

4. Plan of Work



5. METHODOLOGY

5.1 Materials and Methods - Pre Clinical

Table 5.1.1: The ingredients, anatomical parts used and their quantities in CKC

S.No	Ingredients	Part used	Quantity
1	Nerunjil (*Tribulus terrestris* Linn)	Fruit	35gms
2	Nilapanai (*Curculigo orchioide* Gaertn)	Rhizome	35gms
3	Murungai (*Moringa oleifera* Lam)	Seed	35gms
4	Poonaikaali (*Mucuna prurita* Hook)	Seed	35gms
5	Iluppai poo (*Madhuca longifolia* Linn)	Flower	35gms
6	Bhumi chakkarai (*Maerua arenaria* Hook)	Root tuber	35gms
7	Seerakam (*Cuminum cyminum* Linn)	Fruit	35gms
8	Lavangabathiri (*Cinnamomum tamala* Nees)	Leaf	35gms
9	Lavangapattai (*Cinnamomum verum* Presl)	Stem Bark	35gms
10	Kirambu (*Syzygiumaromaticum* Linn)	Flower bud	35gms
11	Elavampisin (*Bombax ceiba* Linn)	Gum	35gms
12	Drakshai (*Vitis vinifera* Linn)	Fruit	35gms
13	Koshtam (*Costus speciosus* Koen)	Root	35gms
14	Athimathuram (*Glycyrrhiza glabra* Linn)	Root	35gms
15	Sirunagappo (*Mesua ferrea* Linn)	Flower	35gms
16	Perichankai (*Phoenix dactilifera* Linn)	Unripe fruit	35gms
17	Moongil uppu (*Bambusaaurundinaceae* Willd)	Salt	35gms
18	Jaathikkai (*Myristica fragrans* Houtt)	Seed	35gms
19	Korai kizhangu (*Cyperus rotundus* Linn)	Rhizome	35gms
20	Takkolam (*Ilicium verum* Hook)	Flower	35gms
21	Maramanjal (*Coscinium fenestratum* Gaertn)	Stem bark	17.5gms
22	Aadaathoda (*Adhatoda vasica* Nees)	Seed	35gms
23	Maruthani (*Lawsonia inermis* Linn)	Seed	35gms
24	Ponnakani (*Alternanthera sessilis* Linn)	Seed	35gms
25	Gomutra silasathu (*Asphaltum punjabinum)*	Fine-ash	35gms

Table 5.1.2: Raw Drugs Procurement

Drugs	Drug procurement
Adhatoda vasica	Research Institute for Indian System of Medicine, Himachal Pradesh, India
Alternanthera sessilis	National Institute of Siddha, Tamilnadu, India
Asphaltum punjabinum	SKM- Tamil Nadu, India
Other herbal drugs	Govindhasamy chetty store, Tamilnadu, India

Identification and Authentication of Raw Drugs

Adhatoda vasica seeds were procured from the Research Institute for Indian System of Medicine, Joginder Nagar, Mandi, Himachal Pradesh, India. *Alternanthera sessilis* seeds were collected from the herbal garden, National Institute of Siddha, Chennai, India. Other herbal drugs were procured from Govindhasamy chetty store, Chennai, India. Gomutra silasathu (mineral drug) was procured from SKM, Tamil Nadu,India. Drugs were identified, authenticated and voucher specimen (NIS/MB/59/2012) was deposited in the Department of Medicinal Botany, National Institute of Siddha, Chennai.

Table 5.1.3: Analytical studies conducted - Institutes and Laboratory

Studies conducted	Samples	Institutes and Laboratory
Pharmacognostical study	*Adhatoda vasica* *Alternantherasessilis*	PARC - Plant anatomy research centre, Tamilnadu, India
Phyto chemical analysis	*Adhatoda vasica* seeds *Chandrakanthi chooranam*	
Physico chemical anlaysis	*Adhatoda vasica* seeds *Chandrakanthi chooranam* *Silasathu Parpam*	CCRS [Siddha Central Research Institute], Tamilnadu, India
Chromatographic studies (TLC/HPTLC)	*Adhatoda vasica* seeds *Chandrakanthi chooranam*	
ICP OES analysis	*Chandrakanthi chooranam* *Silasathu Parpam*	
Microbial contamination **Specific-pathogens** **Aflatoxin** **Pesticide residue**	*Chandrakanthi chooranam*	Sargam Laboratory, Tamilnadu, India.
Particle size analysis	*Silasathu Parpam*	
TGA analysis	*Chandrakanthi chooranam*	Department of Chemistry, Indian Institute of Technology, Chennai, India
CHN analysis	*Silasathu Parpam*	

Pharmacognostical study

In literature survey no pharmacognostical studies have been carried out on the seeds of *Adhatoda vasica* Nees, and *Alternanthera sessilis* Linn, hence the study was undertaken for the establishment of proper identification and authentication of the seeds.

Seed Analysis - Macroscopic and microscopic

The analysis was carried out for *Adhatoda vasica* Nees and *Alternanthera sessilis* Linn seeds. Seeds were fixed in FAA solution (5ml of Formalin + 5ml of Acetic acid + 90 ml of 70% ethyl alcohol) for 24 hrs. After that the samples were dehydrated through graded series of the tertiary - butyl alcohol.[197] Infiltration of the samples were carried out by the slow addition of paraffin wax (58-60°C-melting point) till the TBA-solution reach super saturation.The samples were cast in to the paraffin blocks.

Sectioning

The paraffin fixed samples were fragmented with the help of Rotary Microtome. The sections thickness was 10 to 12 µm. De-waxing of the fragmentations were by customary procedure. [198] The fragments were stained by toluidine-blue since because it is a polychromatic-stain. [199] The results (staining) were really good and a few cytochemical-reactions were also found. The dye showed pink (cellulose walls), blue (lignified cells), dark green (suberin), violet (mucilage) and blue (protein bodies) colour. Wherever essential fragments were also stained with IKI (starch), fast green and safranin.

Photo-micrographs

Microscopic explanation of the tissues is enhanced with micrographs where ever required. Photographs with various magnifications were captured with the help of Nikon lab photo 2 -microscopic units. For regular examinations bright field was employed. For crystal study, lignified cells and starch grains polarized light was applied. Since these constitutions contain birefringent property, they emerge brighter against the dark background when viewed through a polarized light. Figure enlargement is specified by scale-bars.[200]

Chemical (Phyto / physico) analysis

Chemical analysis were carried out in *Adhatoda vasica* seeds by the procedures refered in the standard literature. [201, 202, 203]

Purification process of the herbal and mineral ingredients [204,40,82,25]

Primarily all the drugs were purified as per the procedures mentioned in Siddha literature. *Glycyrrhiza glabra, Coscinium fenestratum, Cyperus rotundus and Maerua arenaria* were washed in water, outer skin were peeled off and then dried in the sunlight. Seeds present in *Phoenix dactilifera and Myristica fragrans* were removed, outer portion were dried in sunlight and used.*Curculigo orchioides* was dried, powdered and then par boiled in milk for 1 samam [3hours], then dried under sunlight and then powdered. Impurities of *Costus speciosus, Cuminum cyminum, Mesua ferrea, Bombax ceiba, Cinnamomum verum, Cinnamomum tamala, Ilicium verum, Syzygium aromaticum, Bambusa aurundinaceae Madhuca longifolia, Tribulus terrestris, Vitis vinifera, Moringa oleifera, Adhatoda vasica, Mucuna prurita, Lawsonia inermis* and *Alternanthera sessilis* were removed and dried in sunlight. *Gomutra silasathu* was mixed with the cow's urine and then filtered with a thick cloth. And then dried in the sunlight.Layer was formed on the filtrate (fig 5.1.2A) which was then removed and dried up. This method was repeated until no more layer was formed (7 times).

Preparation of *Gomutra Silasathu parpam* [25]

Gomutra Silasathu parpam (one among the 25 ingredients of CKC) was prepared as per the method demonstrated in the Siddha literature¨ 35gms [1 palam] of the silasathu (purified) was soaked in pulitha arisi kazhuviya neer (fermented rice water) for three days. Fresh fermented rice water was used for each days. On the 4th day, the drug was put on the mortar to triturate with the pulitha kazhuneer for twelve hours (4 samam). Pellets were prepared (fig 5.1.2 B) and dried. Following drying, the pellets were placed in between two earthen pots, positioned one above the other. The earthen pots were sealed with cloth (cotton) smeared by fuller's earth and dried in the sun light. Pellets were then put in to oxidation process (Pudam) with twenty five cow-dung cakes. After ignition, it was permitted to quench itself. The finishing products were taken out, pulverized and then stored in air tight containers.

Preparation of the study drug *Chandrakanthi Chooranam* [25]

All the purified herbal ingredients and *silasathu parpam* was together powdered and shifted in to a 100 size mesh. Chooranam was par boiled with milk (final purification process) then finally dried and stored.

Drug storage

The trial drug was stored in clean and dry wide mouthed bottle. It was kept in room temperature and protected from sunlight.

Analytical studies

Gomutra silasathu parpam

Analytical studies of Gomutra silasathu parpam was carried out to ensure that the parpam added in the study drug CKC is of standard quality.

Siddha specification for parpam [205]

Siddha specifications for parpam was analyzed through parameters specified in the standard siddha manuscript. It was observed for luster in the daylight, since lustre should not be present as per siddha text. Parpam was rubbed between the thumb and the index finger and it must enter in to the lines of fingers. Parpam was sprinkled over still water which was taken in the beaker and it must float on water surface. Little quantity was kept on the tongue and it must be tasteless.

Physico-chemical parameters

Loss on drying (at 105°C), Ash values (total ash; water soluble ash; acid insoluble ash), pH and the particle size were examined by standard methods. [203]

ICP-OES Analysis [206]

Nutritional elements like Potassium; Calcium; Magnesium; Iron ; Sodium; Manganese; Zinc; Copper; Nickel; Selenium; Tellurium; Cadmium were studied.

Particle size

The particle size for Gomutra silasathu parpam was analyzed out in 3 sieve sizes (150 micron, 75 micron and 45 micron) [207]

CHN Analysis

CHN analysis was examined in a PE 2400 series II- CHNSO analyzer; Perkin Elmer; USA.

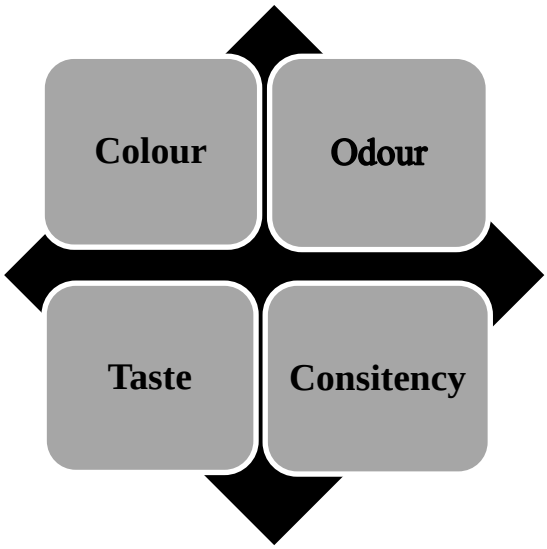
Table 5.1.4: Chromatographical study (HPTLC and TLC)

HPTLC/TLC Methods	***Chandrakanthi chooranam***	***Adhatoda vasica seeds***
Extract preparation	4 gms of the drug was taken and soaked in chloroform in the night. Then boiled in a water bath for about 10 mts which is then filtered and then concentrated to 10-ml.	4 gm of the drug was initialy refluxed with 100-ml hexane, after that it was filtered to eliminate the fatty materials. Process should be repeated with an additional hexane (100ml). Then the filtrate was soaked during the night in the chloroform. Then boiled in a water bath for about 10 mts which is then filtered and then concentrated to 10-ml.
Solvent system	Toluene: Ethyl-acetate - 5:0.5; v/v showed good separation	Toluene: Ethyl-acetate - 10:1.5; v/v showed good resolution
	The appropriate solvent system was attained by the trial and error method. This sovent was employed in developing extract on TLC-plate.	
Volume of the extract (Chloroform)	7, 10 and 12 µl	5, 10 and 15 µl
Visualizing reagent The reagent vanillin sulphuric acid was selected as the visualizing reagent (1gm of vanillin was added in the ethanol: sulphuric acid mixture in the ratio of 95:5) because it gives the colour for most of the secondary metabolites categories.		
Instrument To develop the TLC plate CAMAG- twin chamber was used. Applicator Linomat IV- CAMAG; Muttenz; Switzerland: was used for the extract application. Aluminium plate was precoated through silica gel - $60F_{254}$ which is of 0.2 mm in thickness (Merck) and it was used for TLC plate. Bands width as of 8 and 6 mm of distance in between the tracks were used on the plate (6 x10 cm). For finger print study scanner CAMAG TLC - 030618 which is attached with the WINCATS- software were employed under UV-254 and 366 nm and then after the derivatization at 540 nm. For photo documentation CAMAG-visualizer was applied at UV 254 nm; 366 nm and in the visible lights following to the dipping in the reagent vanillin-sulphuric acid which is then followed with heating in the air circulated oven until the formation of the coloured spots.		
Procedure With the help of the applicator (Linomat IV) the cholroform extract was then applied on TLC plate in the volume of 7, 10 and 12 µl as 8 mm bands by 6 mm space in between the tracks and then developed in the above stated solvent method. This developed plate (TLC) was dried in the air and then photographs were captured under the UV-254 and 366 nm. Plate was then scanned using the scanner under the UV- 254 nm and UV-366 nm. Finger print was documented. Plate was then dipped in the reagent of vanillin-sulphuric acid, which is heated in the oven at the degree of 105°C until the formation of the coloured spots. Photograph was captured immediately and then scanned for finger printing profile at 540nm.		

Chandrakanthi chooranam

Organoleptic characters

Figure 5.1.4: Organoleptic character study in *Chandrakanthi chooranam*



Preliminary Phytochemical Test

The test was studied out as per standard methods mentioned in the literature.[201, 202]

Table 5.1.5: Phytochemical study method

Phytochemical Test	Method	Colourof appearance
Protein (Biuret test)	Sample solution was taken in the test tube and sodium hydroxide solution is added to it. Little drops of very dilute copper II –sulphate (1%) solution was then mixed gently.	Purple colour
Steroids (Lieberman Burchard Test)	In a dry test tube, to the extract (few mg) chloroform (2ml) was added. Then acetic acid (few drops) was added, heated and then acetic anhydride (few drops) and concentrated sulphuric acid (2 drops) were added.	Green colour
Flavonoids (Shinoda test)	Substance was made to dissolve in alcohol and added to bits of magnesium and conc-hydrochloric acid and then heated over a water bath.	Magenta colour
Triterpenoids (Noller's Test)	To the few mg of the extract, tin and thionyl chloride was added and heated in water bath.	Purple colour
Phenol	5 % alcoholic ferric chloride was added to the substance in water	Dark blue/Green colour

Tannin	Sample substance is shaken with the water and then added with lead acetate solution.	White precipitate
Test for Alkaloids (Dragendorff's Test)	Few mg of extract in separate test tube was warmed with 2% Sulphuric acid for 2 minutes. And it was filtered in separate test tube and few drops of Dragendorff's reagent were added.	Orange-red precipitate
Glycosides	Sample substance is added with anthrone and conc- sulphuric acid and then heated in a water bath.	Green colour
Cardiac glycoside (Keller-Killani Test)	To the extract glacial acetic acid (2ml) containing a drop of ferric chloride solution was added and then 0.5 ml of conc-sulphuric acid was added.	Blue colour in the acetic acid layer
Reducing sugar (Fehling's Test)	Fehling's reagent was added to the sample solution.	Brick red precipitate or coloration
Saponin	To the extract (few mg) distilled water is added and then shaken well.	Formation of foam

Physico-chemical parameters

Loss on drying (at 105°C), Ash values (total ash; water soluble ash; acid insoluble ash), water soluble and alcohol soluble extractive; pH and the particle size were carried out as per standard guidelines [203,208]

Table 5.1.6: Physico- chemical study method

Parameters	Apparatus/ Chemicals	Procedure	Calculation
Loss on drying at 105°C (Percentage)	1] Analytical balance range, 0 to 210 g 2] Air circulated oven 3] Beaker – 100 ml	4 g of accurately weighed drug was dried at 105º for 5 hours and weighed. The process of drying and weighing at 1 hr period until the difference between two succeeding weights corresponding to not more than 0.25 per cent was continued. Constant weight was attained when two succeeding weighings after drying for 30 mts and cooling for 30 mts in a desiccator. Showed not more than 0.01 g in difference.	Sample wt losss x 100 Wt of the sample
Total ash (Percentage)	1] Analytical balance range 0 to 210 g 2] Muffle furnace 3] Water bath 4] Silica dish 5] Desiccator	2-3 g accurately weighed, ground drug in a preweighed silica dish was incinerated. The percentage of ash with reference to the air dried drug was calculated	Wt of ash x 100 Wt of the sample

Water soluble Ash (Percentage)	1]Analytical balance, range 0 to 210 g 2] Muffle furnace 3] Silica dish 4]Whatman 41 filter paper 5] Distilled water	Ash obtained from the above test was boiled in 25 ml of distilled water for few mts. One more time the process was repeated. This insoluble matter was filtered on an ashless filter paper and ignited in a silica crucible to constant weight. The percentage of water soluble ash with reference to the air dried drug was calculated	Total ash(wt -water insoluble residue(wt) x 100 Wt of the sample
Acid insoluble Ash (Percentage)	1] Analytical balance, range 0 to 210 g 2] Muffle furnace 3] Silica dish 4] Hydrochloric acid AR 5] Distilled water.	Ash obtained from the previous test was boiled in 25 ml of dilute hydrochloric acid for 5mts. Insoluble matter was filtered on an ashless filter paper, washed with hot water and ignited in a silica crucible to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was calculated	Acid-insoluble residue (wt) x 100 Wt of the sample
Water-soluble extractive (Percentage)	1] Analytical balance: range 0 to 210 g, 2] Water bath 3] Air oven 4] Glass stoppered flask 5] Distilled water(100 ml) 6] Pipette – 25 ml 7] Beaker – 100 ml	5 g of the air dried drug (coarsely powdered) was macerated with 100 ml of the distilled water in a closed flask for 24 hours, shaking often for 6 hrs and allowing to stand for 18 hrs. It was filtered quickly, taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated in a tared flat bottomed shallow dish, and dried at 105°, to constant weight and weighed. The percentage of alcohol-soluble extractive with reference to the air-dried drug was calculated.	Wt of the extractx100x 100 25 x wt of the sample
Alcohol soluble extractive	1] Analytical balance, range 0 to 210g 2] Water bath 3] Air oven 4] Beaker – 100 ml 5] Glass stoppered flask 6] Pipette – 25 ml 7] Distilled alcohol (100ml)	5 g of the air dried drug (coarsely powdered) was macerated with 100 ml of alcohol of the specified strength in a closed flask for 24 hrs, shaking often for 6hrs and allowing to stand for 18 hrs. It was filtered quickly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated in a tared flat bottomed shallow	Wt of the extract x 100 x 100 25 x weight of the sample taken

		dish and dried at 105º, to constant weight and weighed.The percentage of alcohol-soluble extractive with reference to the air-dried drug was calculated.	
Alkalinity	1] Conical flasks 2]Whatman 41 filter paper 3] Distilled water 4]Graduated-pipette10 ml 5]0.1N Hydrochloric acid 6]Phenolphthalein indicator	Few drops of phenolphthalein indicator were added to the filtrate collected from water soluble ash analysis. Titrated against 0.1 N Hydrochloric acid. End point was the disappearance of pink colour. Report the alkalinity per gram of the sample (ml of 0.1N HCl/g of the sample).	

ICP-OES Analysis [206]

Heavy metals (mercury, lead, arsenic and cadmium) and nutritional elements that supports spermatogenesis (Iron, calcium, magnesium, copper, zinc and selenium & zinc) were studied as stated by the standard guidelines. 0.5 g of the study drug (CKC) was accurately weighed and taken in a 250 ml beaker. 15 ml of conc nitric acid and per-chloric acid mixture (taken in the ratio 2:1) and microwave was digested to get the clear solution. Then the solution was filtered in a filter paper (Whatmann No.41) into a standard flask (100 ml) and made equal to the mark using the de-ionized water. This resulting solution was used to study about the elements which was present in the sample of drug. Perkin-Elmer: ICP-OES Optima; 5300 DV outfitted with an AS-93- auto sampler with WinLab3- software was employed for the analysis. Nitrogen was utilized for optical purge gas at the flow speed of 1.4 L / mt and at 365 kPa pressure.

Microbial contamination and specific Pathogens [208]

Microbial contaminations (total bacterial and fungal count) and other specific pathogens (*E. coli; Staphylococcus aureus; Salmonella* spp and *Pseudomonas aeruginosa)* were tested as indicated by standard procedures.

Test for Aflatoxin and Pesticide residue [208]

The mycotoxins/ aflatoxins (B1; B2; G1 and G2) and pesticide residues (organo chlorine; organo phosphoru) were tested as by standard procedures.

Thermo gravimetric analysis

The TGA analysis (thermogravimetric analysis) of CKC was carried out using the TG instrument - TGA Q500 V20.10- Build 36. Quantity of sample (accurately weighed) was heated in a high resolution- nitrogen atmosphere and by maintaining at the rate of 20^0/mts.

Toxicity study (Acute and Long term)

Acute and long term toxicity study was carried out following the World Health Organization (WHO) guidelines [1993] with the minor modifications. [209]

Drug and Dose :

The therapeutic dose for the study drug (CKC) for acute and long term toxicity study was calculated by extrapolating the human-clinical dose (12 gm/day) to rat dose (216mg / 200gm b.wt; 1.08 gm /kg b.wt) which was based on the ratio of the body surface. [210] Drug was made in to suspension by adding with its vehicle milk [2ml] in mortar-pestle. The drug was administered to rats with respect to their individual weights.

Route of administration

Oral route was selected, both for acute and long term toxicity study as it is the clinical route of administration.

Procurement and rearing of experimental animals

Adult male wistar rats weighing 130-220 gms were used for both acute and long term toxicity study. The animals were procured from National Centre for Laboratory Animal Sciences (NCLAS), NIN, Hyderabad. They were housed three per cage under standard laboratory conditions at a room temperature at 20 ± 2^0 C. Ventilated by air conditioning with

100% fresh air and humidity was maintained between 50-70%. The animals were subjected under standard photo-periodic condition of 12:12 hr light dark cycle. The animals were fed with standard rodent pellet procured from M/s. Provimi Animal Nutrition India Pvt Ltd, Bengaluru and purified RO water (Kent RO water filter cum purifier) ad libitum. Animals were acclimatized to laboratory conditions one week prior to the initiation of the experiments. The protocol for experimentation was approved by Institutional Animal Ethics Committee (Ref.no: NIS/IAEC/I/2011/2(A)) of National Institute of Siddha, Chennai, Tamilnadu, India.

Acute toxicity study

Experimental design

Table 5.1.7: Experimental design in acute toxicity study

Sample Size	18 wistar rats
Sex	Male
Route of Administration	Oral
Experiment Duration	14 days
Drug	*Chandrakanthi chooranam*
Dose	10.8 gm/kg/p.o (10 times the dose equivalent to human therapeutic dosage was selected to ascertain its safety potential)

Animal grouping and interventions

The animals were randomly divided into three groups (I, II and III) of six rats (n=6) each. Individual identification of the animal was made by marking. Group I animals served as control and received 10ml/kg b.wt of distilled water. Group II received once with 10ml/kg bwt of milk and served as vehicle control. Group III served as the treated groups and received 10 times the dose equivalent to human therapeutic dose [10.8gm/kg/p.o.] of *CKC*

Table 5.1.8: Animal grouping and intervention in acute toxicity study

Groups	Intervention	No of Rats
Normal Control- Group I	Distilled water	6
Vehicle control - Group II	Milk	6
10 x TD - Group III	*CKC* (10.8g / kg b.wt)	6

In-life observation

Doses were administered to the wistar rats which were overnight fasted with water *ad libitum.* All the rats were observed for general conditions, signs of toxic symptoms and mortality for every hour during the first day with particular concentration given during the first 4 h and thereafter every day for 14 days. Parameters such as mortality, allergic reactions,

skin colour changes, response to handling, secretions, pilo-erection, posture, gait, diarrhoea, tremors, sleep, convulsion signs, circling, depression, sedation, excitement and cyanosis were observed and then recorded.

Physiological parameters

Feed and water consumptions were recorded daily. Individual body weight of the wistar rats were recorded previous to the dosing, on the 7th day and prior to the sacrifice on the 14th day.

Gross necropsy

After the observation period of 14 days, all surviving rats were sacrificed and were subjected to the complete gross necropsy to examine any signs of systemic-toxicity. External surface of the body, cranial, orifices, thoracic and the abdominal cavities and its contents were examined. Lastly, the vital organs like heart, lungs, liver, kidneys, spleen, brain and testis was grossly examined.

Long term Toxicity

Experimental design

Table 5.1.9: Experimental design in long term toxicity study

Sample Size	30 wistar rats
Sex	Male
Route of Administration	Oral
Experiment Duration	As a rule of WHO-guidelines for the clinical administration of drug, between 1-6 months, the toxicity study administration period is from 3- 6 months and as a result 3 months [90 days] was selected as the treatment schedule.
Drug	*Chandrakanthi chooranam*
Dose	Three dose levels were studied to produce the range of toxic effects and mortality rates. ▶ **Therapeutic dose:** 1.08gm/kg b.w of *CKC* ▶ **Average dose (3 x TD):** 3.24g/k g b.w. of *CKC* ▶ **Higher dose (5 x TD):** 5.4 g / kg b.w. of *CKC*

Animal grouping and interventions

The wistar rats were randomly divided into five groups (I, II,III,IV and V) of six rats (n=6) each. The groups of animals were transferred in to different cages and were marked for their identifications. Group I animals served as control and received 10ml/kg b.wt of distilled water. Group II received 10ml/kg b.wt of milk and served as vehicle control. Group-III rats received *CKC* at a dose equivalent to human therapeutic dose (1.08gm/kg/p.o), Group-IV rats received 3 times the dose equivalent to human therapeutic dose (3.24g/k g/ p.o.) of *CKC*. Group V rats received 5 times the dose equivalent to human therapeutic dose (5.4gms/kg b.wt) of *CKC*.

Table 5.1.10: Animal grouping and interventions in long term toxicity study

Groups	Intervention	No of Rats
Normal Control - Group I	Distilled water	6
Vehicle control - Group II	Milk	6
1x TD - Group III	*CKC* (1.08 g / kg b.wt)	6
3 Xtd - Group IV	*CKC* (3.24g / kg b.wt)	6
5 x TD - Group V	*CKC* (5.4 g / kg b.wt)	6

Sampling, Sacrifice and Surgical procedure:

Twenty-four (24) hours after the 90^{th} day of treatment, following over-night fasting (12 h) blood was collected from the rats through retro orbital- plexus into 2 tubes, one contains EDTA for the analysis of haematological parameters and the other in to a sterile plain bottle without additives wich was centrifuged at 4000 rpm at 4◦C for 10 mts to get the serum for biochemical estimations. The resulting samples was obtained and stored at −20 °C prior to the biochemical estimation. After this all the wistar rats were sacrificed with the excess anaesthesia. The abdominal cavity was opened via a midline incision.The organs like heart, lungs, spleen, kidneys, liver, testis, brain, stomach and thymus were excised and examined for gross lesions and weighed. Representative tissue samples were preserved in 10% formalin solution for histopathological evaluation.

Pathological Evaluation

Final body weight, organ weight, blood picture, blood biochemical markers and histological examination of internal organs were determined.

Physiological parameters

Daily consumption was measured by calculating difference between the amounts of food and water given and their remnants on the next day. The body weights of all rats were recorded [gm] before and after the session [90days] of each respective drug administration (before sacrifice).

Organ weights

Organs like heart, lungs, spleen, kidneys, liver, testis, brain, stomach and thymus were dissected out, freed from the surrounding fats and connective tissues. Mopped with the tissue paper and was weighed (absolute). Organs were weighed on the digital balance soon after the dissection to avoid drying [wet weight were recorded].

Blood chemistry- panel

Hematological Assays

Variables were analyzed using automated bayer- hematology analyzer which includes total white blood cell count (WBC), lymphocytes (LYMP), Monocytes (MONO), Granulocytes(GRAN), Total red blood cell count (RBC), platelet count (PLT), platelet crit (PCT); mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), haemoglobin (Hb), Hematocrit (HCT) or packed cell volume (PCV), red blood cells width (RDW), Platelet distribution width (PDW), mean platelet volume (MPV)

Biochemical Assays

Biochemical variables like glucose, urea, creatinine, total cholesterol, triglycerides (TGL), total Protein (TPN), albumin, globulin, total bilirubin, Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were determined using chemical analyzer RA 50. Electrolyes were analyzed by Transasia electrolyte analyzer-EC lyte transasia.

Histopathological study

The tissues examined were: Heart, lungs, spleen, intestine, kidneys, liver, testis, brain and thymus. Tissue samples were preserved in formalin (10%) for histopathological evaluation. The tissue samples were usually embedded into paraffin; 2-m thick sections were stained with H & E (hematoxylin and eosin). All the sample slides were examined microscopically for any pathological observations.

Pharmacological study

Spermatogenic activity

Chemicals

Chemicals used in this study were obtained from the Sigma Chemicals Company, U.S.A. Further analytical grade- chemicals were procured from the S.d. Fine Chemicals Ltd., Mumbai, India.

Drug and Dose

The therapeutic dose for the study drug (CKC) for acute and long term toxicity study was calculated by extrapolating the human-clinical dose (12 gm/day) to rat dose (216mg / 200gm b.wt; 1.08 gm/kg b.wt) which was based on the ratio of the body surface. [210] Drug was made in to suspension by adding along with its vehicle milk [216mg of *CKC* in 1ml of milk]. The drug was administered to rats with respect to their individual weights.

Procurement and rearing of experimental animals

Adult male wistar rats weighing 180-210 gms were used for this study. The in-bred animals were procured from the animal house of C.L. Baid Metha College of Pharmacy, Chennai, India. They were housed three per cage under standard laboratory conditions at a room temperature at 22 ± 2^0 C. The animals were subjected under standard photoperiodic condition of 12:12 hr light dark cycle. The animals were fed with standard rodent pellet procured from Avian feed farms, Bangalore and water ad libitum. Animals were acclimatized to the laboratory conditions a week prior to the initiation of experiment. The protocol for experimentation was approved by the IAEC- Institutional Animal Ethics Committee (Ref.no:IAEC/XLI/01/CLBMCP/2013) of C.L.Baid Mehta College of Pharmacy, India.

Experimental design

Table 5.1.11: Experimental design in spermatogenic study

Sample size	18 wistar rats
Sex	Male
Route of Administration	Oral
Experiment Duration	60 days
Drug	*Chandrakanthi chooranam*
Dose	1.08 gm / kg b.wt

Animal grouping and interventions

The animals were randomly divided into three groups (I, II and III) of six rats (n=6) each. Individual identification of the animal was made by marking. Group I animals served as normal control and received 1ml double distilled water for 60 days. Group II received 0.5 ml of ethanol (25%) /kg /b.wt /day and served as negative control (alcohol induced).[98] Group III served as the treated group and received 0.5 ml of ethanol (25%) /kg/b.wt along with 1.08g/kg of *CKC* for 60 days. Administration was done once daily by oral gavage in the morning (between 7.30a.m – 9.00 a.m)

Body and genital organ weights

The body weights of all wistar rats were recorded [gm] before and after the experiment [60days]. The wistar rats were sacrificed on the next day after the last day of drug administration [61st] and the reproductive organs like testes, prostate, seminal vesicle and epididymis were dissected out and freed from surrounding fat and connective tissue and weighed on a Cubis Precision Balance for absolute weight.

Table 5.1.12: Animal grouping and interventions in spermatogenic study

S. No	Intervention	No of Rats
Normal Control -Group I	Distilled water	6
EthanolInduced-Group II	25% of ethanol (0.5 ml /kg/b.w/day)	6
Treatment -Group III	*CKC*chooranam (1.08 g / kg b.w / day) + 25% of ethanol (0.5 ml /kg/b.w/day)	6

Sampling, Sacrifice and Surgical procedure

Twenty-four (24) hours after the 60th day of treatment, following over-night fasting(12hrs), the wistar rats were anaesthetized under ether anaesthesia and the blood samples were collected into clean dry centrifugal tubes by the carotid bleeding with the aid of a 21G needle. The blood was allowed to stand for about 15mts to clot and was further spun in a centrifuge at 1000g for 10 mts. Serum was separated from the clot with Pasteur pipette into the sterile sample tubes. The serum samples obtained was stored at −20°C prior to the estimation of total protein, total cholesterol and testosterone. After this all the wistar rats were sacrificed with i.p. (intraperitonial) injection of thiopentone. The abdominal cavity was opened up through a midline incision to expose the genital organs. Testes, prostate, seminal vesicle and epididymis were excised, all the fats were trimmed of and the tissues were wiped with the tissue paper and then weighed. Left testicles were collected to examine the sperm

characters and the right testicles to examine testicular and epididymal histopathological study. The sections were studied microscopically for histo architecture changes. The left caudal- epididymis were transferred into a sterile bottle which contains 2 ml of the normal saline for semen examination. Semen samples from the caudal epididymis (left) were tested for the parameters such as count, motility, viability and morphology. Counting was performed through haemocytometer and light microscope with 100X

Blood chemistry- panel

Estimation of serum total cholesterol

Span diagnostic kit was applied for the estimation of total cholesterol, which followed cholesterol oxidase/peroxidase (CHOD-POD) method. [211]

Table 5.1.13: Reagents used

Reagent composition	Conc. in the final test mixed
Good's butter (PH 6.7)	50mmol/l
Phenol	5mmol/l
4 - Aminoantipyrine	0.3 mmol/l
Cholesterol esterase	>200 U/l
Cholesterol oxidase	>100 U/l
Peroxidase	>3 KU/l
Standard: The concentration of standard glucose used was 200mg/dl	
Assay &Procedure: Fresh clear and unhaemolysed serum was used for the evaluation.	

Table 5.1.14: Reaction Parameters

Reaction type	End point
Wavelength	505 nm
Optical path	1 cm
Temperature	370°C
Measurement	Against reagent blank

Table 5.1.15: Summary of assay details

Pipetted in to test tube	**Blank**	**Standard**	**Test**
Reagent	1000 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Sample	-	-	10 µl

The reaction mixtures were mixed up well and then incubated for 10 mts at 370°C. The absorbance of the reaction mixtures at 505nm against reagent blank was taken. The absorbance was calculated by using a Shimadzu spectro photometer (model 1601).

Calculation

$$\text{Total cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc. of standard.}$$

Estimation of total protein

The amount of total protein was evaluated using standard kits employing the methods of Lowry using folin phenol reagent (Lowry *et al)* [212]

Estimation of glycogen content

The supernatant sample (0.5ml) were subjected to the alkali digestion with KOH (30%) in boiling water-bath for 20mts. 3 ml of the ethanol was added and the tubes were kept in a freezer overnight. They were centrifuged at 3000-rpm for 40 mts. The precipitate was dissolved in warm water, re-precipitated with ethanol and then centrifuged again. The final precipitate was dissolved in 3 ml of distilled water and heated for 5 mts in a boiling water-bath. Aliquots of the sample were mixed with 4 ml of anthrone reagent, heated in a boiling water-bath for 20 mts. Green colour developed was studied at 600 nm with utilizing Systronics UV VIS- spectrophotometer. The glycogen content in the tissues was expressed as mg. [213]

Estimation of serum testosterone

Serum testosterone was evalutaed by the ECLIA method using standard test kit [Rache – German]. Assays were carried out according to the manufacturer's instructions.

Enumeration of sperm parameters

Semen analysis

Examination of sperm count, motility, viability and morphology was carried out by making small cuts in the area of the cauda-epididymis which was close to vas deferens and then applied gentle pressure to exude epididymal contents.

Sperm Count [214]

Semen sample was drawn in to WBC pipette and diluted to the ratio of 1:100 with the modified Krebs Ringer-bicarbonate buffer containing 0.05% collagenase (pH 7.4). After this 1:1000 dilution was carried out with NaCl (1.8%) and formalin (2%). Sperm suspension was placed in the haemocytometer with improved double Neubauer ruling, which was used for the counting of the sperms. Counts for 2-4 haemocytometer chambers were averaged.[214]

Sperm count evaluation

- Total number of sperm cells in all the four chamber = X
- X multiplied by 10,000 to obtain the number of cells (Y) per ml of diluted sample
- Y multiplied by 100 (the dilution factor) to obtain (Z) sperm cells per ml of original semen sample.

Sperm Motility [214]

The sample was mixed with 20mM HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid contains L-Glutamine with BSA- Bovine serum albumin (5%). Final sample suspension was mixed with formalin and was used to examine motility using the bright field microscope with the magnification of 100 x

$$\textbf{Motility (\%)} = \frac{\text{Number of motile spermatozoa}}{\text{Total number of spermatozoa (motile + immotile)}} \times 100$$

Percentage viability [215]

Sperm suspension was diluted with trypan blue solution (0.4%) in the ratio of 1:1 dilution and then was pipetted up and down many times to make sure the uniform cell suspension with the use of same pipette tip and was allowed to stand for 5 to 15 mts. Small amount of the trypan blue-cell suspension was transferred in to the hemocytometer chambers carefully. The cells were viewed under a microscope at the magnification of 100 x. Live sperm appeared colourless with bright margin under contrast and dead cells stained blue and are non-refractile.

$$\textbf{Percentage of sperm viability:} \quad \frac{\text{Total Viable cells (Unstained)}}{\text{Total cells (Viable +Dead)]}} \times 100$$

Sperm morphology [216]

Staining

1mL of sperm suspension was transferred in to a test tube. 1% eosin Y (2 drops) was added to a test tube and then mixed by gentle agitation. Spermatozoa were incubated at the room temperature for approximately 45 to 60 mts and allowed for staining.

Slide preparation

Slides was cleaned up with the detergent and washed in water and then in alcohol, dried before use. 1-2 drops of the stained sperm suspension was placed approximately one cm from frosted end of the pre cleaned microscope-slide lying on the flat surface. Second slide was held on the right hand with long edge of the slide that gently touches across the width of the sperm slide and was pulled across to make a sperm smear. Smear was allowed to dry and then was fixed with the formalin.

Characterization of normal and abnormal sperm

Sperm abnormality was observed based on the subsequent parameters like headless tail, tail less head, curved tail, coiled tail and looped tail. Normal sperm was observed based on appearance and the absence of above refered parameters.

Percentage of normal = No of normal sperm / Total number of sperms in the filed X 100
Percentage of abnormal = No of abnormal sperm / Total number of sperms in the filed X 100

Procedure for histopathology

The rats from each group were sacrificed with i.p. (intraperitonial) injection of thiopentone . The abdominal cavity was opened via a midline incision and the genital organs were exposed. Right testicles was excised. The testicle samples were washed with the normal saline and then fixed in neutral formalin (10%) for 48 hrs for further histological observation. Paraffin sections were taken at the thickness of 5 μm and then processed in the alcohol-xylene series and then was stained with the haematoxylin-eosin dye. The section was examined microscopically for any histopathological changes. The magnification at 10 X (low power) and 45 X (high power) was carried out. [217]

5.2. Patients and Clinical Methods

Clinical study was carried out in to two phases

- Pilot study
- Main Clinical Trial

Pilot study

This study was designed to the assess the feasibility, safety and tolerability of administration of study drug CKC in Oligozoospermic patients prior to conducting phase II main clinical trial. Study was conducted in the out patient department of National Institute of Siddha, Chennai, India. Out of initially screened 21 male patients aged between 21-45 years, 16 patients were excluded as they could not meet up the inclusion criteria and 5 patients were enrolled for the trial. Only those who gave the written consent were included in the study. Method followed was similar to that of the main clinical study. Separate case record forms were maintained for each patients. All subjects were closely followed for adverse effects related to the treatment. The obtained datas were subjected to statistical analysis and the safety and efficacy of the treatment were validated

Main Clinical Trial

Table 5.2.1: Study design

Study population	Infertile male, age-21-45yrs, low sperm count
Study type	Open label, safety and efficacy study, Phase-II, Interventional
Study centre	National Institute of Siddha
Allocation	Non- Randomized
Intervention	Single group assignment
Study period	1year
Sample size	40

Clinical Trial Registration

The Trial was registered in Clinical Trials Registry (India) with the registration number CTRI/2014/01/004281 and registered in Clinical Trials.gov with the registration number NCT01847963.

Ethical Clearance

The study protocol was approved by the Ethical Committee of National Institute of Siddha [NIS/IEC/2011/1/12]

Clinical source

Male patients of 21 to 45 years old presenting with the complaint of primary or secondary infertility, visiting out patient department of National Institute of Siddha, Chennai, India, who were diagnosed as oligozoospermia with count lower than 15 million sperm cells / ml.

Selection Criteria

Table 5.2.2 Inclusion criteria

- Male infertile patients with age between 21-45 years
- Marriage history for >1 year
- Sperm count 1-15 million/ml [below one million is excluded]
- Patients with normal liver & renal function test
- Willing to give specimen of semen before & at the end of the clinical trial
- Informed patients giving written consent

Table 5.2.3: Exclusion criteria

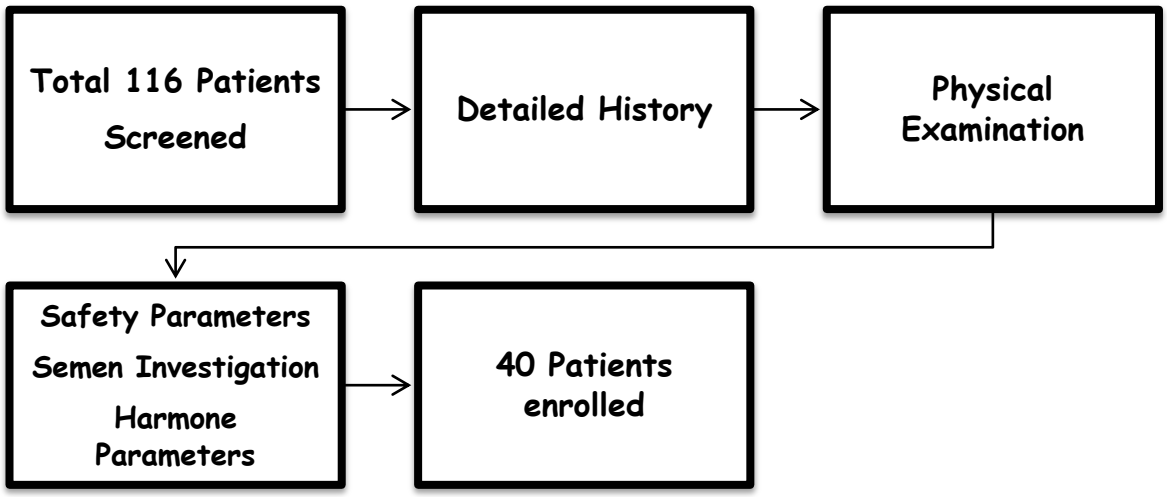
- Azoospermia-complete absence of sperm in the ejaculate
- Aspermia-complete lack of semen
- Necrospermia- spermatozoa in semen are dead
- Clinical diagnosis of varicocele & hydrocele
- History of undescended testis
- Inguinal hernia on physical examination
- Male accessory gland infection
- History of DM, Hypertension and Cardiac Disease
- Any recent medical or surgical illness
- Underwent treatment for promoting spermatogenetic fertility in last 3 months
- Other systemic disease requiring specific therapies
- Known Thyroid disease
- Past history of renal, hepatic or any other chronic illness in the patient

Screening examinations

Male patients who had a history of primary and secondary infertility for a period of more than 1-years were initially selected for the study. Detailed history was taken regarding previous illness, infections or surgery. Physical examination was carried out to rule out any endocrinological disorder or genital abnormality. Semen examination and safety parameters like blood glucose, haematological test, renal function tests, liver function tests, lipid profile, VDRL test and urine routine test were performed. Thyroid test, seminal culture, scrotal ultrasonography and antisperm-antibodies were also evaluated in patient with suspected pathology. A total of 116 male patients aged between 21-45 years were initially screened

from April to October 2013, from the out patient department (OPD) of National Institute of Siddha. 40 patients were enrolled for the clinical trial. Only those who gave written consent were included in the study.

Figure 5.2.1 Screening Examination of Patient



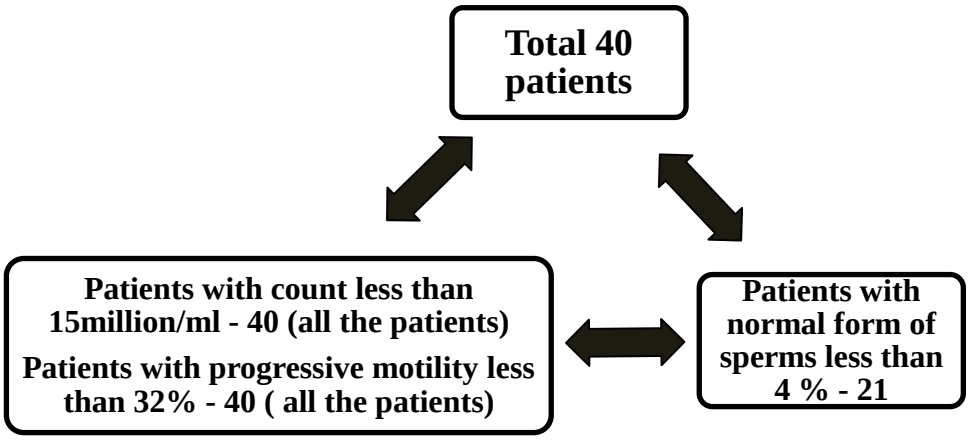
Informed consent

Consent from each subject was obtained in response to a fully written and verbal explanation of the nature of the study before the start of the trial. The details were given in two languages (Enlish and Tamil). Patients were free to withdraw their name from the study at any time without giving any reason.

Selection of patient

A total of 40 eligible men from totally screened 116 men with fertility problems were diagnosed as Oligozoospermia on the basis of semen analysis and fulfilling the inclusion criteria were registered for the clinical study.

Figure 5.2.2: The selected Oligozoospermic Patients (40) comes under two categories



Investigations

Parameters were assessed at baseline (Day 0), at the end of the trial (Day 91) and at the end of the follow up (day181). Semen analysis and all the other safety parameters were performed at same Institution laboratory. Harmone analysis were assessed at Hitech Diagnostic center, Tamilnadu, India.

A] Semen examination

Semen examination were performed in order to diagnose and assess the effect of the therapy.at World Health Organization (WHO) recommended standards. [49] Rapid analyses were performed within one hour after collection. Macro and microscopic assessment of the semen were carried out to measure the semen volume [ml], liquification time [minutes], semen viscosity [high/low], sperm concentration [million/ml], percentage of progressive and total motile spermatozoa, percentage of normal forms of sperm and pus cells (per field). The seminal fluid characteristics were assessed twice before the registration of each patient and both at least one week apart.

Table 5.2.4: Collection of semen

Abstinence	5 days
Method	Mastrubation
Container	Dried and wide mouthed bottle with 50ml capacity
Place	A private room adjacent to the Institute laboratory
Collection time	Between 9.00 AM to 12.00 PM

B] Harmone Analysis

Serum samples were measured for testosterone and follicle stimulating hormone (FSH) by ECLIA method and luteinizing hormone (LH) by CLIA method.

C] Safety parameters

i] Hematological Analysis:

Hb (gm/dl), Total RBC (cells/µl), Total WBC count (million/µl)), Erythrocyte sedimentation rate (ESR) and Platelets (lakhs /µl)

ii] Biochemical Analysis

Blood Glucose(mg/dl), serum total cholesterol (mg/dl), HDL (mg/dl), LDL(md/dl), VLDL (mg/dl), serum triglycerides (mg/dl), blood urea (mg/dl), serum creatinine (mg/dl), uric acid (mg/dl), serum total bilirubin (mg/dl), serum total protein (gm/dl), AST (IU/ml), ALT (IU/ml) and alkaline phosphatase (IU/L).

iii] **Urine routine**

Albumin, Sugar, bile salts, bile pigments, Urobilinogen, Acetone and occult blood.

D] **Siddha Investigations**

Envagai thervu (eight fold examination), *Neer Kuri* (examination of urine) and *Nei Kuri* [oil on urine sign] [32]

Line of Treatment

***Viresanam* [Purification of Alimentary Tract]**

The Selected patients had been given *Agasthiar Kuzhambu* at the dose of 130 mg with ginger juice in the early morning in empty stomach to bring the *mukutram* to equilibrium.[218]

Table 5.2.5: Interventional Medicine [25]

Drug: *Chandrakanthi chooranam* **Posology:** 12gm –OD; Bed time **Route of Administration:** Oral route **Administration period:** 3 months (90 days) ***Anupanam* (Adjuvant):** Milk (150ml)

Table 5.2.6: Pathiyam and Apathiyam [30, 40, 36]

Pathiyam **(Diet and behaviors to follow)**	
Diet	Greens - (Drumstick, Climbing brinjal, Spinash, *Amaranthus tritis*) Goat meat; Emperor and Eel fish Plantain flower, Drumstick, Mango fruit, Black grapes, Black plum, Pomegranate Cow's milk, Cashew, Almond and Walnut
Behaviour	Oil bath twice in a week (avoid intercourse on the day of the oil bath)
Apathiyam **(Diet and behaviors to avoid)**	
Diet	Avoid Horse gram, Mango, Bitter guord and Sesban leaves
Behaviour	Avoid intercourse in day time and during digestion of the food
Other Advices	
Avoid tobacco, smoking, alcohol, drug abuse, hot baths, strenuous activities, occupation in hot environment, wear loose under wear and control obesity	

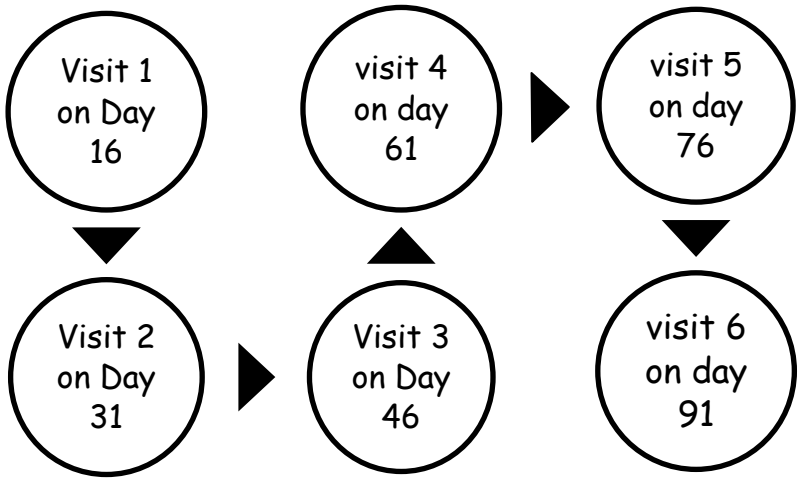
Conduct of the study [219, 220]

- The study was conducted in accordance with the guidelines of the Indian Council for Medical Research (ICMR) and GCP adapted from ICH and accepted by Ayush.
- The study was conducted for one year in between April 2013 and April 2014 in the out patient department of National Institute of Siddha, Chennai, India.
- After recruitment, patients were put on 90 days of interventional Medicine
- To take uniform quantity of medicine all the patients were given 15 zip lock cover (1 zip lock cover containing 12gms of *CKC)* of *CKC* in every trial visit.
- Patients were advised to take the trial drug appropriately. They were advised to follow the appropriate diet mentioned by the investigator. The patients were instructed to bring unused drug on next visit. The details were recorded in drug compliance form.
- After completing three months duration and follow up period, patients were instructed to take investigations concerning sperm parameters, harmone parameters and safety parameters.

Trial Visits

During the treatment period of 90 days, the subjects were required to present themselves at the trial center at precised intervals: Visit 1 on Day 16; Visit 2 on Day 31; Visit 3 on Day 46, visit 4 on day 61, visit 5 on day 76, and visit 6 on day 91.

Figure 5.2.3: Trial Visits of Patients



Case record form (CRF)

Patients were given unique registration number. The screening forms were filed separately. History of present and past illness, personal history, family history, habits, physical examination findings, subjective parameters, objective parameters, investigations, drug compliance, dietary advice, informed consent and adverse events were systemically recorded in the CRF for analysis. The case taking proforma was elaboratively designed for the purpose of incorporating Siddha method of diagnosis as mentioned in the classics. After enrolling the patient in the study, a separate file for each patient was opened and all forms were be kept in that file.

Assessment criteria

A] Objective parameters

Sperm concentration, progressive and total motile spermatozoa, normal forms of sperm, testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH)

B] Subjective parameters

i] Clinical Symptoms

Erectyle dysfunction, premature ejaculation and nocturnal emmision were recorded.

ii] Siddha clinical parameters

Envagai thervu (eight fold examination), uyir thathukal (bio energetic principle) and udal thathukal (physical constituents) were assessed.

Follow up

After the completion of treatment the patients were kept under follow up, for the period of three months and were then assessed for the overall effect of the therapy if any. Follow up records were further documented in the CRF.

Adverse Effect

The safety and tolerability of study medications was assessed based on adverse effects reported by patients or observed by the investigator during evaluation. Every 15^{th} day the patients were assessed for clinical improvement and adverse effects. In case of adverse effects either reported or observed in the course of study period the patient will be advised to stop the medicine and it will be documented in the adverse effect form in CRF and will be intimated to the Pharmacovigilence committee of NIS

Withdrawal Criteria

Table 5.2.7 Patients Withdrawal Criteria

Any adverse effects reported
Patients failed to strictly adhere to the study protocol
Patients turned unwilling to continue the clinical trial

In the case of adverse effect reported, the trial drug was stopped and withdrawal form was filled up in CRF and then the patient was treated in OPD of NIS. In case of emergency, the patients were referred to nearby Government hospital for management.

Observation criteria

Contemporary variables

Age, nature of work, educational status, Socio economic status, sexual habits, sleeping pattern, diet, addiction, infertility, duration of infertility, pscychological status, gonadotoxic agents, previous illness, family history, blood group, bathing habit, BMI, semen parameters, clinical parameters, harmone parameters and safety parameters were assessed.

Siddha variables

Kaalam [Patients age], *Thinai* (Habitat), *Noi utra kaalam* (season), *Thega* ilakanam (bio type), *Udal thathukal* (physical constituents), *Envagai thervu* (eight fold examination),

Naadi nadai (Pulse play), *Uyir thathukkal* (Functional constitution of the body) *and* status of *agni* (basal metabolic heat) were assessed.

Outcome measures

Primary outcome measures

Semen parameters were graded into four groups depending upon the response to *CKC* as follows: a) Good, b) Moderate c) Mild and d) Poor

Table 5.2.8: Primary outcome measures

Grading	Sperm count (million)	Progressive Motility (Percentage)	Total Motility (Percentage)	Normal Forms (Percentage)
Good	>30	> 40	> 50	> 10
Moderate	20-30	32- 40	40 – 50	4- 10
Mild	2-19	1-31	1-39	1-3
Poor	no changes	no changes	no changes	no changes

Secondary outcome mesaures

Changes in the level of serum testosterone, FSH, and LH

Compliance

- As per ICMR guidelines, Phase II is a therapeutic exploratory trials and normally 20 - 25 patients should be studied for assessment of each dosage. [8]
- In this study 37 patients have completed the trial out of estimated 40 registered patients and their data were analyzed.
- Three patients dropped out from the study prior to completion for the reasons: of withdrawal (n=2); discontinuation (n=1).

Data collection and Management

The datas were kept securely, access was granted for the purpose of the study and the confidentiality of the patients was maintained. Datas of subjective parameters, objective parameters and safety parameters collected at baseline, at the end of the treatment and at the end of the follow up were taken out from the case record form and were computed in the MS Excel software and was considered for statistical analysis. Demographic datas, disease related datas, *Siddha* classic investigations and diagnostic methods were collected from the CRF and

was properly analyzed. After sorting the data, a calculation was performed to obtain the percentage of subject with the individual factor and were documented. The Data entries were monitored by the guide and all the analysis were done by the Investigator.

Statistical Analysis

Analysis were done by one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test. All the statistical analysis was carried out using SPSS Software version 19. Datas were expressed as mean ± standard error of the mean (SEM). The datas obtained from toxicity studies were analyzed using Student's *t*-test to compare the treatment groups with control group.The datas obtained from spermatogenic study were analyzed using Student's *t*-test to compare the treatment group with control and induced group.The datas obtained from clinical study was analyzed using Student's *t*-test to compare the values from the baseline to the end of the treatment and end of the follow up. For toxicity study significance levels was set at $*p < 0.05$ and $**p < 0.01$ and for spermatogenic study significance levels was set at $*p < 0.05$ and $***p < 0.001$ and for clinical study significance levels was set at $*p < 0.05$; $**p < 0.01$ and $***p < 0.001$ [Main clinical trial and Pilot study].

6. RESULT

Pharmacognostical study

Alternanthera sessilis

Macroscopical characters of the seed

The seeds were dark brown in colour (fig 6.1a, 6.1 b) with absence of odour, shape is of elliptical, with broad blunt end and opposite end being short conical in shape (fig 6.2). The length of the seed is 400µm and wide is 250 µm.

Microscopical characters of the seed

The testa (seed-coat) was hard and thick. The thickness was 10µm and the thickness was uniform all through the seed. Outer surface was uneven with ridges being irregular. Outer surface of the testa (seed coat) had 2 or 3 prominent wings or appendages (fig 6.3). The length of the wings was 80µm and the thickness was 30µm. The wings were multicellular and with thick wall. The shape was conical. The testa (seed coat) comprises of sclerotesta or outer sclerotic epidermis and with inner seed coat (which was also sclerotic). There are 2 or 3 layers of the fibrous cells. (fig 6.3, 6.4). Endosperm was thin walled, large and with polyhedral outline. (fig 6.5, 6.6).

Adhatoda vasica

Macroscopic characters

Seeds were brown, flat, elliptical-oblong and fairly thick. Seeds were rugose and sub orbicular. It was measured 6 by 5mm. A prominent- median ridge runs starting from base to top (fig 6.7).

Microscopic characters

Seed comprises of thin seed coat- testa and a pair of plano-convex thick cotyledons. Testa consisits of outer-sclerotesta (sclerotic seed coat) and inner-sarcotesta (parenchymatous part). Highly undulate sclerotesta with the regular-ridges and furrows all through seed surface (fig 6.8) were observed. Thin sarcotesta which includes the outer thin walled and large cell layer (parenchyma cells) and inner - thin layers with thick- walled cells. Parenchymatous

zone was thin at lateral part of seed which was developed into wider; many-layered at chalazal end (fig 6.9, 6.10). Sarcotesta cells comprises of inclusion of the dark cells. Sclerotesta which was greatly folded into the ridges and wide-furrows has outer and the inner tangential- wall. Radial walls are spindle shaped and were thickened (fig 6.11, 6.12). And the thick radial-walls were lignified. The thickness of the sclero testa was 40 mm and of the whole testa was 150 µm. Radicle was circular in the sectional and the diameter is 550 µm. It comprises of the procambial strand and young meristematic-tissue. Cotyledons were of plano-convex and with the flat surface facing to each others. The cotyledons cells were of parenchymatous and includes thick starch grains (fig 6.11, 6.8).

Powder Microscopy

The seed coat fragments were seen in the surface view. Outer epidermis is sclerotesta that appears to be ameboid in the outline since because of the thick and liquefied wavy - anticlinal walls. Lumen of the cells was wide with variable outline (fig 6.13, 6.14). Sarcotesta cells are parenchymatous (inner zone). Cells are observed in small fragments and are polyhedral within the outline. Cell walls being thick with pits. Cells have thick inclusions which are amorphous and dark stained (fig 6.15). Broken pieces of cotyledons were commonly present in the powder. Cells were rectangular or squarish. Cell walls were straight and thick. Starch grains were abundantly present in the cells. The size of the cells was 20 to 30 x 10 to 15 nm (fig 6.16). The cotyledonary cells were also found in the solitary conditions. They were rectangular- squarish or with triangular outline. Cells were darkly stained (fig 6.17). Spherical and shinning OB (oil bodies) of different sizes was seen abundantely in the powder and they don't stain by safranin. Oil bodies were smaller to large in size and were found to be free floating on water medium (fig 6.18). Spherical and large SG (starch grains) were sparingly seen and they found to stain dark to IKI. The grains were mainly concentric with central-hilum and the diameter is up to 70 µm (fig 6.19).

Physico-chemical parameters

Loss on drying (at 105^0C) was 6.8 %.; total ash was 4.07 %; acid insoluble ash was 0.15 % ; water soluble extractive was 27.73 % and alcohol soluble extractive was 19.45 % .(Table 6.1).

TABLE 6.1: Physico-chemical analysis of *A. VASICA* Seeds				
Physico chemical Parameters (%, w/w)		**First**	**Second**	**Mean**
Loss on Drying (at 105°C)		6.9	6.7	6.8
Ash values	**Total Ash**	4.07	4.09	4.07
	Acid- soluble Ash	0.15	0.15	0.15
	Water- insoluble Ash	1.75	1.80	1.78
Extractives values	**Alcohol Soluble**	19.8	19.1	19.45
	Water Soluble	27.40	28. 05	27.73

Phytochemical analysis

Phytochemical results showed the presence of flavonoid, alkaloid, phenol, triterpene, saponin, coumarin, steroid, glycosides and tannin. Anthraquinone was found to be absent. (Table 6.2)

Table 6.2 Phytochemical Results of *A. VASICA* seed

Phytochemical Test	**Results**
Flavonoid	Present
Alkaloid	Present
Phenols	Present
Triterpene	Present
Saponin	Present
Coumarin	Present
Steroid	Present
Glycosides	Present
Tannin	Present
Anthroquinone	Absent

TLC/ HPTLC RESULTS

- TLC photo-documentation at UV 254 nm demonstrated 3 spots at the R_f values - 0.03; 0.50 and 0.89, at UV 366 nm demonstrated 4 spots at the R_f values - 0.06; 0.50; 0.58 and 0.89 and after the derivatization by vanillin-sulphuric acid demonstrated 4 spots at the R_f values - 0.05; 0.53; 0.67 and 0.89. (fig 6.20-A,B,C; Table 6.3).

Table 6.3: Spots colour at R_f values under pre and post derivative conditions

UV 254 nm		**UV 366 nm**		**After Derivatization by Vanillin-Sulphuric acid**	
R_f- value	**Spot colour**	**R_f- value**	**Spot colour**	**R_f - value**	**Spot colour**
0.03	Green	0.06	Pale Blue	0.06	Violet
0.50		0.50		0.53	
0.89	-	0.58		0.67	
-		0.89		0.89	

- The finger print study (HPTLC) of *A. vasica* seed at UV- 254 nm demonstrated 9 peaks; at R_f - values 0.19- 11.85%; 0.63- 44.54 % and 0.81- 21.91 %; which are the major peaks. All other spots at the R_f - values 0.10; 0.24; 0.47; 0.70 and 0.75 were minor peaks. 3-D chromatogram study at UV- 254 nm of 3 tracks showed the proportional increment in the height of the peak. (fig 6.21A, 6.21B , Table 6.4).

Table 6.4: R_f values and percentage peak area of the chloroform extract of *Adhatoda vasica* seed at - UV 254 nm

Peak	Start	Start	Max	Max	Max	End	End	Area	Area
	Rf	Height	Rf	Height	%	Rf	Height		%
1	0.09	0.6	0.10	16.0	5.08	0.12	0.3	183.0	3.57
2	0.16	0.6	0.19	22.5	7.18	0.22	8.5	607.3	11.85
3	0.22	8.7	0.24	13.0	4.14	0.27	3.1	308.5	6.02
4	0.46	4.0	0.47	29.0	9.24	0.48	6.6	189.0	3.69
5	0.60	8.9	0.63	168.0	53.47	0.66	13.6	2281.3	44.53
6	0.69	9.2	0.70	11.6	3.69	0.72	3.2	216.0	4.22
7	0.74	2.5	0.75	18.1	5.75	0.76	11.3	215.6	4.21
8	0.78	11.6	0.81	35.9	11.4	0.86	1.4	1122.8	21.91

- HPTLC - finger print study of *A. vasica* seed at - UV 366 nm demonstrated only 2 peaks at the R_f 0.38 - 7.31 % and 0.65 - 92.69 %. 3D chromatogram study at - UV 366 nm of all the 3 tracks are presented. (Table 6.5 (fig 6.22A, 6.22B)

Table 6.5: R_f values and percentage peak area of the chloroform extract of *adhatoda vasica* seed at - UV 366 nm

Peak	Start	Start	Max	Max	Max	End	End	Area	Area
	Rf	Height	Rf	Height	%	Rf	Height		%
1	0.37	1.4	0.38	29.2.	25.37	0.39	1.5	155.4	7.31
2	0.60	1.5	0.65	85.8	74.63	0.69	2.5	1969.7	92.69

- HPTLC- finger print study of *A. vasica* seed at - 540 nm after the derivatization through vanillin-sulphuric acid demonstrated 7peaks at the R_f values - 0.07; 0.09; 0.15; 0.49 - 80.79 %); 0.55; 0.61 - 10.28 % and 0.81- 1.21 %. 3D chromatogram study at 540 nm of all the 3 tracks are presented (Table 6.6 (fig 6.23A, 6.23B).

Table 6.6: R_f values and percentage peak area of the chloroform extract of *Adhatoda vasica* seed at - UV 540 nm

Peak	Start	Start	Max	Max	Max	End	End	Area	Area
	Rf	Height	Rf	Height	%	Rf	Height		%
1	0.06	0.1	0.07	15.8	2.83	0.08	1.7	151. 1	0.42
2	0.09	2.0	0.09	12.3	2.19	0.11	0.5	111.6	0.31
3	0.14	1.0	0.15	11.4	2.05	0.16	5.3	141.6	0.40
4	0.24	5.6	0.49	258.5	46.30	0.54	118.7	28751.6	80.79
5	0.54	118.9	0.55	153.8	27.54	0.57	32.8	2342.1	6.58
6	0.57	33.0	0.61	87.8	15.73	0.66	38.6	3658.5	10.28
7	0.78	1.5	0.81	18.7	3.35	0.84	1.2	431.5	1.21

Analytical study of *Gomutra silasathu parpam*

Siddha Specifications

The dark colour, lusterless, entering in to the lines of the fingers, floating over the still water, tasteless and odourless were observed and the results are mentioned. (Table 6.7, fig 6.24)

Table 6.7: *Siddha* specification for *Parpam (Gomutra Silasathu)*

Specification	**Results**
Colour	Dark colour
Taste	Tastless
Luster	Lusterless
Smell	Odourless
Over the surface of water	Floats
In the lines of the finger	Entered in to the lines of the index and thumb finger

Physico-chemical parameters

GSP (Gomutra silasathu parpam) was examined in the duplicates for analyzing loss on drying (at 105^0C), total ash; water soluble and acid insoluble-ash and pH of the 10% solution.

Table 6.8: Physico-chemical analysis of *GSP (Gomutra Silasathu Parpam)*

Physico chemical Parameters (% ; w/w)	**First**	**Second**	**Mean**
Total ash	77.30	77.60	77.45
Acid insoluble Ash	21.6	20.3	20.45
Water soluble Ash	23.6	23.9	23.75
Loss on Drying (at 105°C)	2.30	2.20	2.50
pH	6.04		

ICP-OES Study

The analysis exposed the presence of K (potassium), Ca (calcium), Mg (magnesium), Fe (iron), Na (sodium), Mn (manganese), Zn (zinc), Cu (copper), Cr (chromium) and Ni (nickel). Their quantity was revealed to be 156261; 32039; 21739; 17318; 12470; 320; 185; 107; 28.6 & 19.2. Se (Selenium), Te (tellurium) and Cd (cadmium) were revealed to be BDL (below detection level) which are shown in table 6.9.

Table 6.9: ICP-OES analysis of Gomutra Silasathu parpam for trace elements

Elements	Quantity- in ppm
K (potassium)	156261
Ca- Calcium	32039
Mg (magnesium)	21739
Fe (Iron)	17318
Na (Sodium)	12470
Mn (Manganese)	320
Zn (Zinc)	185
Cu (Copper)	107
Cr (Chromium)	28.6
Ni (Nickel)	19.2
Se (Selenium)	BDL
Te (Tellurium)	BDL
Cd (Cadmium)	BDL

BDL: Below Detection level ; D.L: Detection Limit; D.L.1.0

Particle Size Analysis

Particle size study was done in 3 sieve sizes - 150; 75 and 45 microns. The analysis demonstrated that the *GSP* accomplish its fineness.

Table 6.10: Particle size of GSP

Sieve- size (IS sieve)	Inference
150 micron	Passes completely
75 micron	82.46 %
45 micron	68.79 %

CHN analysis

CHN- analysis results of GSP (Gomutra silasathu parpam) is showed in table 6.11. It revealed the percentage (%) of carbon to be 12.31; hydrogen to be 0.55 and nitrogen to be 1.21

Table 6.11:CHN analysis of GSP

Analysis Parameters	Inference (%)
Carbon (C)	12.31
Hydrogen (H)	0.55
Nitrogen (N)	1.21

Analytical study of *Chandrakanthi choornam*

Organoleptic characters

CKC (Chandrakanthi choornam) is brown coloured- fine powder with the spicy odour. Taste is found to be sweet, astringent and slightly bitter.

Table 6.12: Organoleptic characters of CKC

Characters	Results
Colour	Brown
Taste	Sweet,astringent and slight bitter
Odour	Spicy
Consistency	Fine powder

Preliminary phytochemicals

Phytochemical study showed the presence of steroids, amino acids, phenols, tannins, flavonoids, saponins, anthraquinones, glycosides Triterpenoids and alkaloid was found to be absent.

Table 6.13: Phytochemical results of CKC

Phytochemical Test	Inference
Steroid - Lieberman Burchard's Test	Present
Amino acids - Biurette's test	Present
Phenol	Present
Tannin	Present
Flavonoids - Shinoda's test	Present
Saponins	Present
Anthraquinones	Present
Glycosides	Present
Triterpenoids - Noller's Test	Present
Alkaloids - Dragendorff's Test	Absent

Physico-chemical parameters

The drug CKC passes through mesh size of 100. The inference were 8.45 percentage of loss on drying (at 105°C); 13.04 perecntage of total ash; 3.39 percentage of water soluble

ash; 5.61 percentage of acid insoluble ash; 4.04 perecentage of acid soluble ash; 19.25 percentage of water soluble extractive; 16.85 percentage of alcohol soluble extractive and pH value of 6.37.

Table 6.14: Physico-chemical parameters of CKC

Physico-chemical Parameters (%)		**First**	**Second**	**Mean**
Loss on Drying (at 105°C)		8.591	8.325	8.458
Ash values	**Total Ash**	13.074	13.011	13.043
	Acid insoluble Ash	5.788	5.434	5.611
	Water soluble Ash	3.244	3.539	3.392
Extractive values	**Alcohol Soluble**	16.8	16.9	16.85
	Water Soluble	19.20	19.30	19.25
pH		6.37		
Particle size		Passes completely through 100 size mesh		

ICP-OES Analaysis

Heavy metals (mercury, lead, cadmium andarsenic) were found to be DL (below detection level - 0.05 ppm). The quantity of nutritional elements like Ca (calcium), Mg (magnesium), Fe (iron), Zn (zinc) and Cu (copper) were estimated to be 6482.9; 1870; 988.6; 21.98 and 8.09 ppm in that order. The quantity of calcium was estimated to be high. Mg and Fe were observed to be moderate comparatively. Zn and Cu were identified to be in lesser amount

Table 6.15: Heavy metal analysis of CKC

Heavy metals	**AYUSH/WHO/FDA Specification**	**Inference**
Cd (Cadmium)	0.3 ppm	BDL
Pb (Lead)	10 ppm	
Hg (Mercury)	1 ppm	
Ar (Arsenic)	3.0 ppm	
BDL (Below Detection level) ; DL (Detection Limit) -0.05 ppm		

Table 6.16: ICP-OES analysis of CKC

Elements	**Quantity - ppm**
Mg (Magnesium)	1870.0
Ca (Calcium)	6482.9
Zn (Zinc)	21.98
Cu (Copper)	8.09
Fe (Iron)	988.6
Se (Selenium)	BDL
BDL (Below Detection level) ; DL (Detection Limit)	

Thin Layer Chromatography

The TLC- photo documentations of CKC (at UV 254 nm; UV 366 nm and after derivatization) were presented in fig 6.25. R_f- values and spots colour under UV 254 nm; UV 366 nm; after derivatization were shown in the table 6.17. TLC photo documentation of CKC (Chandrakanthi choornam) at UV 254 nm revealed 5 visible green spots at the R_f value - 0.19; 0.14; 0.24 – major; 0.43 and 0.75. At UV 366 nm showed 8 spots at the R_f - 0.09 - pale blue; 0.23 - greenish blue; 0.32 - greenish blue; 0.41- greenish blue; 0.48- greenish blue; 0.54 - pale blue; 0.59 - blue and 0.65- greenish blue. TLC plate- after derivatization (with vanillin sulphuric acid) revealed seven spots at the R_f - 0.05 – purple; 0.17 – purple; 0.38- purple; 0.44- purple; 0.58- purple; 0.69- bluish purple and 0.96 - purple.(fig 6.25)

Table 6.17: R_f values and spots colour of Cholroform extract of CKC

UV 254 nm		**UV 366 nm**		**After Derivatization**	
R_f -value	**Spots colour**	**R_f- value**	**Spots colour**	**R_f - value**	**Spots colour**
0.09 0.14 0.24 0.43 0.75	Green	0.09	Pale blue	0.05	Purple
		0.23 0.32 0.41 0.48	Greenish blue	0.17 0.38 0.44 0.58	
		0.54	Pale blue	0.69	Bluish Purple
		0.59	Blue	0.96	Purple
		0.65	Greenish blue		

High Performance Thin Layer Chromatography

The HPTLC- finger printing of $CHCl_3$ extract of *CKC* at UV- 254 nm (fig 6.26) revealed seven peaks at the R_f 0.09; 0.14; 0.24; 0.31; 0.43; 0.54; 0.75. The percentage (%) area of respective peaks were - 9.58; 22.11; 39.00; 3.64; 17.43; 1.38 and 6.87 in corresponding order. Table 6.18 reveals the R_f values and the percent (%) peak area of the peaks at UV- 254 nm. HPTLC- finger printing of $CHCl_3$ extract of *CKC* at UV 366 (fig 6.27) revealed nine peaks at the R_f - 0.09; 0.12; 0.23; 0.32; 0.41; 0.48; 0.54; 0.59 ; 0.65. The percentage (%) area of the peaks were- 2.83; 1.67; 3.00; 19.96; 27.45; 36.22; 4.50; 2.41 and 1.96 in the corresponding order. Table 6.19 revealed the R_f- values and the percent (%) peak area of the peaks at UV 366-nm. HPTLC - finger printing of $CHCl_3$ extract of *CKC* after derivatization at 540 nm (fig.6.28) revealed eleven peaks at the R_{f-} 0.15; 0.36; 0.44; 0.54; 0.67; 0.72; 0.76; 0.78; 0.81; 0.87; 0.90. The percentage (%) area of the peaks were 3.37; 21.66; 18.42; 6.16; 16.98; 17.97; 1.73; 1.68; 0.66; 3.74; 7.63 in the respective order. The Table 6.20 revealed R_f-values and the percent (%) peak area of the peaks after derivatization at 540nm.

Table 6.18: R_f values and percent area of all the peaks of CKC under UV-254 nm

Peak	Start	Start	Max	Max	Max	End	End	Area	Area
	Rf	Height	Rf	Height	%	Rf	Height		%
1	0.07	0.3	0.09	52.4	11.16	0.10	46.6	1049.8	9.58
2	0.11	47.5	0.14	98.4	20.98	0.16	0.1	2423.7	22.11
3	0.20	1.4	0.24	184.1	39.25	0.28	1.3	4274.7	39.00
4	0.28	1.5	0.31	16.3	3.47	0.33	9.1	398.5	3.64
5	0.40	7.6	0.43	84.3	17.97	0.49	6.5	1910.5	17.43
6	0.53	9.8	0.54	11.9	2.55	0.55	0.4	151.3	1.38
7	0.71	6.2	0.75	21.7	4.63	0.79	0.5	752.9	6.87

Table 6.19: R_f values and percent area of all the peaks of CKC at UV-366 nm

Peak	Start	Start	Max	Max	Max	End	End	Area	Area
	Rf	Height	Rf	Height	%	Rf	Height		%
1	0.07	0.7	0.09	21.5	4.26	0.10	8.1	383.1	2.83
2	0.11	7.8	0.12	16.7	3.31	0.14	1.9	225.9	1.67
3	0.20	0.6	0.23	0.23	2.90	0.26	0.2	405.5	3.00
4	0.27	0.1	0.32	0.32	17.89	0.36	14.2	2700.9	19.96
5	0.36	14.3	0.41	0.41	21.20	0.45	11.7	3713.6	27.45
6	0.45	11.8	0.48	0.48	41.73	0.52	10.6	4901.4	36.22
7	0.52	10.9	0.54	0.54	4.31	0.57	8.8	609.2	4.50
8	0.57	8.9	0.59	0.59	2.24	0.62	1.3	325.8	2.41
9	0.63	0.7	0.65	0.65	2.15	0.67	4.9	265.3	1.96

Table 6.20: R_f values and percent area of all the peaks of CKC at UV-540 nm

Peak	Start	Start	Max	Max	Max	End	End	Area	Area
	Rf	Height	Rf	Height	%	Rf	Height		%
1	0.12	0.5	0.15	23.0	4.11	0.17	0.9	470.2	3.37
2	0.30	9.8	0.36	59.9	10.73	0.40	39.5	3023.5	21.66
3	0.41	39.6	0.44	95.1	17.02	0.48	6.7	2571.1	18.42
4	0.50	2.7	0.54	24.1	4.31	0.59	3.2	860.2	6.16
5	0.62	3.3	0.67	84.9	15.19	0.69	1.4	2371.1	16.98
6	0.71	19.7	0.72	151.5	27.10	0.75	6.8	2508.5	17.97
7	0.75	7.6	0.76	21.7	3.88	0.77	13.8	242.1	1.73
8	0.77	14.0	0.78	14.7	2.64	0.80	0.6	234.0	1.68
9	0.80	0.9	0.81	11.6	2.07	0.82	0.2	91.6	0.66
10	0.84	2.5	0.87	19.4	3.47	0.89	0.6	521.8	3.74
11	0.89	0.1	0.90	53.0	9.48	0.93	27.0	1065.7	7.63

Microbial contamination and specific pathogens

The bacterial count and the fungal count were observed to be within the specified limits. The specific pathogens (*Salmonella* spp*, E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) were known to be absent. The inferences were tabulated in the table 6.21.

Table 6.21 Microbial contamination and specific Pathogens results of CKC

Microbial Test	Inference	AYUSH/WHO/FDA -Specification
Total Fungal count	305 CFU/g	NMT 10^3 CFU/g
Total bacterial count	17,000CFU/g	NMT 10^5 CFU/g
Salmonella pp	Absent/g	Absent/g
E. coli		
Staphyloccus aureus		
Pseudomonas aeruginosa		

Aflatoxins / mycotoxins and Pesticide Residues

Aflatoxins / mycotoxins (B1; B2; G1& G2) were observed to be BDL (below detection limit). Pesticide residues (organo chlorine /organo phosphorus) were found to be not detected in CKC. Results of aflatoxins and the pesticide residues were shown in the table 6.22

Table 6.22- Aflatoxins / mycotoxins and Pesticide Residues

Aflatoxins and Pesticide Residues	Inference
Aflatoxin B1,B2,G1 and G2	BDL (DL: 0.3 μg/kg)
Organo phosphorus /organo chlorine	Not detected

(DL: 0.005 mg/kg)

Thermo gravimetric analysis:

TGA- spectra of CKC (fig 6.29) revealed peaks at- 120^0C; 235^0C; 390^0C; 910^0C. At the degree of 120 centigrade (120^0C) 5.148 percentage of the drug CKC decomposes. At the degree of two hundred and thirty five centigrade (235^0C) 12.78 percentage of the drug CKC was decomposed / disintegrated. Likewise at 390^0C; 41.29 percentage of the drug CKC was disintegrated and at the 910 degree centigrade, 13.43 perecentage of the drug CKC was disintegrated.

Toxicity study

Effect on feed, water intake and survival of wistar rats

No abnormal changes was observed in the feed and water intake of wistar rats between the control and treated groups in both acute and long term toxicity study.No mortality were observed in both acute and long term toxcity and the survival was hundred percent.

Acute Toxicity study

The study drug CKC treated rats at 10 TD dose level.did not show any death, behavioural changes and toxic signs immediately after dosing, during 14 days and at the end of the trial.On necropsy, no gross pathological abnormalities were observed in the vital organs (fig 6.30A, 6.30B) and hence the toxicity of the drug at 10 TD dose level can be ruled out.

Body weight gain

Weight gain (body) of vehicle control group showed significant ($P<0.05$) increase and 10 TD group showed non significant changes when compared to that of the control group. (Table 6.23, 6.24, fig 6.30)

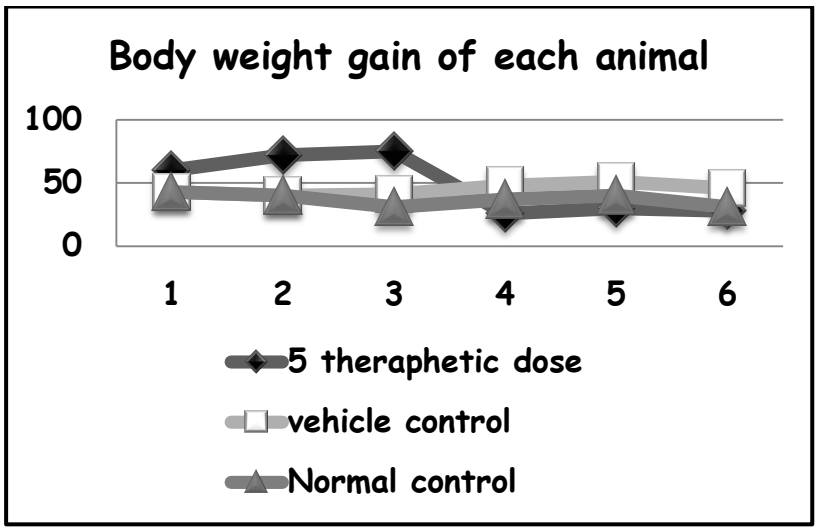
Table 6.23: Final body weight gain (mean) of each animal in acute toxicity study

Groups	**Male Wistar Rats- Weight gain in gms**					
	1	**2**	**3**	**4**	**5**	**6**
Normal control	43	40	31	37	40	31
Vehicle control	43	40	41	48	51	46
5 TD CKC	60	72	75	26	30	28

Table 6.24: Final body weight gain of each group in acute toxicity study

Groups	**Body weight change in (g)**
Normal control	37 ± 2.0490
Vehicle control	44.83 ± 1.7400*
5 TD CKC	48.5 ± 9.4080
Values are expressed as mean ±S.E.M; *P<0.05	

Figure 6.31: Final body weight gain (mean) of each animal in acute toxicity study



Long term Toxicity study

Body Weight Gain

Body weight gain of vehicle control, 3 TD ($P < 0.05$) and 5TD ($P<0.001$) group showed significant increase than the control group. TD group showed non significant changes ($P > 0.05$) in the body weight gain.(table 6.25, 6.26, fig 6.31)

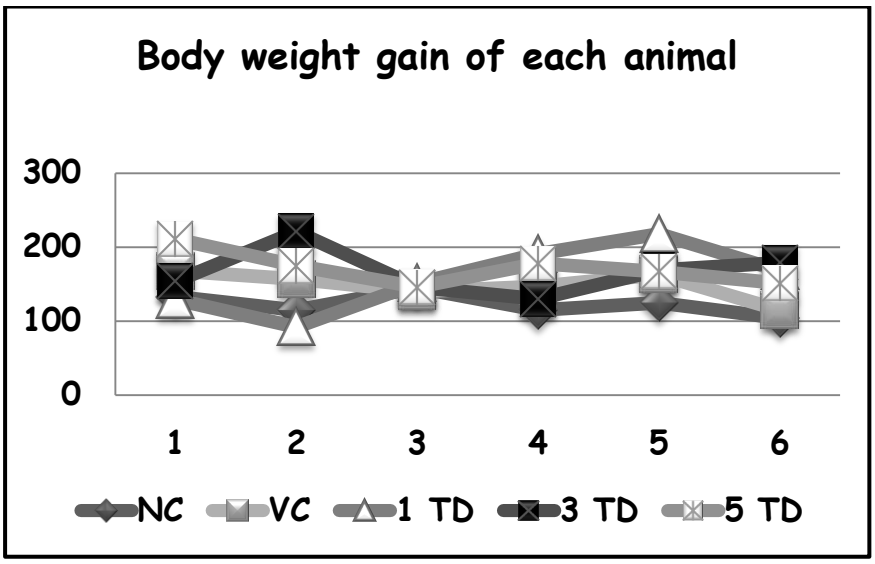
Table 6.25: Final body weight gain (mean) of each animal in long term toxicity study

Groups	Male Wistar Rats- Weight gain in gms					
	1	**2**	**3**	**4**	**5**	**6**
Normal control	133	115	145	115	125	104
Vehicle control	168	157	140	147	163	115
1 TD CKC	129	92	151	191	218	168
3 TD CKC	154	221	146	130	169	179
5 TD CKC	211	175	146	178	167	151

Table 6.26: Final body weight gain of each group in long term toxicity study

`Groups	**Body weight change in (g)**
Normal control	122 ± 5.9910
Vehicle control	148 ± 7.8730*
1 TD CKC	158 ± 18.2890
3 TD CKC	166 ± 12.9630*
5 TD CKC	171 ± 9.4960***
Values are expressed as mean ±S.E.M; *P<0.05; ***P<0.001	

Figure 6.32: Final body weight gain (mean) of each animal in long term toxicity study



Hematology

Values of Hb; RBC; WBC; differential counts; MCH; MCHC; Platelets; MPV; PLT; PCT; HCT of the male wistar rats treated with the study drug of all doses levels (TD, 3TD, 5TD) showed non-significant changes when compared to the control group. MCV value of the vehicle control group -showed significant increase ($p < 0.05$) (within normal laboratory limits). The results do not indicate any serious pathological conditions. (Table 6.27)

Clinical Chemistry

- The values of trigycerides; globulin; bilirubin, sodium and blood sugar of wistar rats treated with the three dose levels (TD, 3TD and 5TD) showed non significant changes ($P > 0.05$) when compared with that of the control groups.
- Blood urea was found to be significantly decreased ($P < 0.05$) in TD group which was comparable to the normal control groups and showed non significant changes in vehicle, 3TD and 5TD group.
- Serum creatinine was found to be significantly increased ($P < 0.05$) in vehicle and TD group (within normal limits); and in 3TD and 5 TD groups showed non sinificant changes ($P > 0.05$).
- Total cholesterol showed non significant changes ($P > 0.05$) in 5TD group; significant increase in vehicle ($P < 0.05$), 3TD group ($P < 0.05$) and TD ($P < 0.01$) group (within normal limits).

- Total protein was increased significantly (P< 0.05) in 5TD group and showed non significant changes (P >0.05) in vehicle, TD and 3TD group.
- Albumin was increased significantly (P< 0.05) in 5TD and showed non-significant changes (P >0.05) in vehicle, TD and 3 TD groups (within normal range).
- AST was increased significantly in 3TD (P< 0.05) and vehicle group (P< 0.01) and non significant changes (P >0.05) in TD and 5TD group.
- ALT showed significant decrease (P< 0.05) in 3TD group and non significant changes
- (P >0.05) in vehcile, TD and 5 TD groups.
- Potassium showed significant decrease (P< 0.01) in 5TD group and non significant changes (P >0.05) in vehcile, TD and 3 TD groups.
- Chloride showed significant decrease in (P< 0.01) vehicle, (P< 0.05) 3TD and (P< 0.05) 5TD group and non significant changes (P >0.05) in TD group. All were within normal limits**.** (Table 6.28)

Table 6.27: Effect of CKC on hematological parameters in wistar rats

Parameters	**Normal control**	**Vehicle control**	**1 TD CKC**	**3 TD CKC**	**5TD CKC**
Hb	9.1 ± .7358	12.3±1.0327	10.9±.4879	10.4± 1.4652	10.1±.7055
RBC	5.7± .4317	7.8 ± .6669	7.0 ± .3363	6.6 ± .9262	6.4 ± .4446
WBC	7.7± 1.1870	9.083 ± 1.0167	6.2±.4757	7.0±1.2028	7.3±1.0920
LYM	56.31 ± 2.1911	56.85± 1.3076	56.68± .9816	52.33± 1.6368	58.31±1.1083
MON	3.7±.1962	3.9± .3981	4.0 ±.1310	3.8 ± .3842	4.0 ± .1803
GRAN	39.9± 2.0241	39.2 ± 1.4098	39.2± .9290	43.7 ± 1.3686	37.8 ±1.1545
MCV	46.13± .4455	47.93±.3989*	46.16±.3148	46.21±.5023	46.71± .2960
MCH	15.78±.2182	15.68 ±.1759	15.30±.1915	15.46±.0919	15.98 ±.1249
MCHC	34.383± .3458	32.85±.4794	33.30±.2769	33.58± .3563	34.2 ±.2887
PLT	230.50±16.707	277± 28.931	233.67 ±15.549	323.33± 63.566	257.33 ±15.794
MPV	5.683 ±.0401	5.750 ± .0671	5.700 ±.0683	5.967 ±.0919	5.700 ±.0365
PCT	.13050 ±.009	.15933 ±.0184	.13250 ± .007	.19333±.039547	.14750±.0081
HCT	26.367 ±2.0089	37.583 ±3.2963	32.700 ±1.5360	31.000 ±4.5195	29.717 ±2.2198
RDW	12.033 ±.4310	11.517 ±.3311	11.650 ±.1688	12.350 ±.2110	11.750 ±.1432
PDW	14.583± .0833	14.733±. .0615	14.767 ±..0760	14.867 ±..0989	14.667 ±..0333

Values are expressed as mean ±S.E.M; *P<0.05

Table 6.28: Effect of CKC on biochemical parameters in wistar rats					
Parameters	**Normal control**	**Vehiclecontrol**	**1 TD CKC**	**3 TD CKC**	**5TD CKC**
Glucose	96.17 ± 7.472	110.83±16.660	80.50±4.660	79.67±5.942	90.33±3.739
Urea	35 ± 1.317	29.17 ± 4.183	28.83 ± 1.558*	30.17 ±3.124	35 ± 4.575
Creatinine	0.6±.0365	0.7 ±.0428*	0.8±.0365*	0.5±.0885	0.7±.1014
Cholesterol	63.33 ± 6.200	84.33± 4.645*	95.17±9.958**	83.17±5.388*	76.11±16.105
Triglycerides	96.50±14.843	123.50± 6.206	135.33±13.583	103.33 ±9.032	132.67±16.760
Protein	7.6± .2887	7.0 ±.1926	7.0 ±.3983	7.8 ±.6019	10.35 1.1147*
Albumin	3.2±.2692	3.2±.1116	3.2±.1470	3.3±.2500	4.3±.2257*
Globulin	3.7±.1478	3.7 ±.2044	3.7±.4301	4.5±.5426	5.9 ±.9807
Bilirubin	0.3± .0333	0.5±.0931	0.4±.0703	0.5± .1195	0.4 ±.1057
AST	136.6±3.774	154± 7.317**	138.50 6.412	173.83±14.481*	160.67±17.416
ALT	63.50± .992	74 ±9.274	60.17± 2.798	53.33± 3.593*	57.17 ±3.114
Sodium	134.33± 1.085	133.33± .803	133.50±.619	132.83±.654	133.83 ±.477
Potassium	7.0±.000	7.6± .3117	7.4 ±.1910	6.6± .2473	7.3 ±.0957**
chloride	106.83±.792	104.33±.494**	105.17± .477	104.33± .494*	104.67± .333*
Values are expressed as mean ±S.E.M; *P<0.05 ; ** P< 0.01					

Organ Weights

Table 6.29: Effect of different dose levels of CKC on organ weight [mg] in wistar rats

	Normal control	**Vehicle control**	**1 TD CKC**	**3 TD CKC**	**5TD CKC**
Brain	1.60 ± .0719	1.76 ± .0585	1.52± .0650	1.56± .0290	1.62 ± .0785
Heart	1.01± .0369	0.97± .03337	0.94 ± .0682	0.91± 0.0463	0.9 2± .0428*
Lungs	1.77±. 0.1173	1.34 ±. 0.0683	1.46 ±0.0440	1.57± 0.1183	1.39± 0.0726*
Liver	10.13 ± 0.3979	8.47 ± .3597	8.91± 0.5843	8.68 ± 0.7400	9.68± .3103
Stomach	1.41± 0.1244	1.44 ± 0.1064	1.45 ± .0263	1.46 ± 0.0444	1.48 ± .0292
Thymus	0.43 ± 0.0445	0.38 ± 0.0230	0.37 ± 0.0111	0.32 ± 0.0329	0.33 ± 0.0403
Testis	2.75 ± 0.0839	2.83 0.0612	2.84 ± 0.1359	2.86± 0.0469*	2.89 ± 0.0549
Kidney	1.31± 0.0852	1.19 ±0.0486	1.2 1± 0.0930	1.10 ± 0.0535	1.12 ± 0.0441
Values are expressed as mean ±S.E.M; *P<0.05					

Absolute weights of brain, liver, stomach, kidney and thymus were found to be comparable with those of the control group rats. Increase in absolute weight of testis ($p < 0.05$) was noted in animals of 3 TD group. Absolute weight of heart was found to be

significantly decreased ($p < 0.05$) in 5TD group and absolute weight of lungs was found to be significantly decreased ($p < 0.05$) in 5TD and vehicle group. (Table 6.29)

Histopathology

Vital organs like brain, heart, liver, kidney, spleen, lungs, stomach, intestine, thymus and testis from both treated (1TD, 3TD, 5TD) and control (normal and vehicle) group animals showed normal architecture. Mild focal chronic gastritis was observed in glandular part of the stomach in one animal of 5TD group (1/6). [Table 6.30, fig 6.33]

Table 6.30: Effect of CKC on histopathological study in wistar rats

Samples	Groups	Male Rats (n=6)
Brain, Heart, Lungs, Liver, Thymus, Spleen, Intestine, Kidney, Testis	Normal control, vehicle control 1TD / 3TD / 5TD	Normal
Stomach	Normal control, vehicle control 1TD and 3TD group	Normal - 5/6
	5TD group	1/6-chronic gastritis
1TD- 1 Therapeutic dose; 3 TD -3 Therapeutic dose ; 5TD -5 Therapeutic dose		

Spermatogenic Activity

Body Weight Changes

Ethanol treated group showed significant decrease ($p< 0.001$) in the mean body weight changes of the rats when compared to that of the control group. Significant increase ($p< 0.001$) in mean body weight changes was observed in study drug group when compared to ethanol induced group. [Table 6.31]

Table 6.31: Effect of CKC on Body weight of rats in Spermatogenic study)

Groups	Initial body weight (gm)	Final Body weight (gm)
Normal Control [NC]	182± .47679	196.78±1.8675
Induced Group [IG]	180.75± .2997	151±.4782 *** a
Treatment Group[TG]	181.80± .3464	190.46±.5725 *** b & c
Values are expressed as mean ±S.E.M; *P<0.001		

Reproductive organ weights

Significant reduction (P<0.001) in the mean values of the weight of epididymis and prostate was noted in ethanol treated group when compared to control group and the mean value of the weight of testis of the ethanol group showed non significant reduction when compared to control group. Study drug treated group showed significant increase ($p < 0.001$) in the weight of testis, epididymis and prostate when compared to ethanol induced group.

Table 6.32: Effect of CKC on reproductive organs of male wistar rats

Groups	Testis (g)	Prostate (g)	Seminal Vesicle (g)
Normal Control [I]	1.57±.0644	0.54±.0148	0.56±.01355
Induced Group [II]	1.43±.0185 a	0.30±.0204 a***	0.42±.0053 a***
Treatment Group[III]	1.52±.0056 b*** ; c	0.49±.0025 b***; c*	0.53±.0051 b/c***

Values are expressed as mean ±S.E.M, n=6, a- Group I vs Group II, b-Group II vs. Group III, c - Group I vs Group III; ***P < 0.001

Biochemical parameters

Cholesterol

Significant increase in mean value of cholesterol ($p < 0.001$) was noted in the ethanol group when compared to that of the control group rats. Study drug group showed significant decrease ($p < 0.001$) in the mean value of cholesterol when compared to the ethanol induced group and is not too far from the control group value. [Table 6.33]

Glycogen

Significant decrease ($p < 0.001$) in the mean values of glycogen was observed in the induced group than the control group. The mean value of glycogen of the study group showed high significant increase ($p < 0.001$) than both ethanol induced and normal control group. [Table 6.33]

Protein

Ethanol induced group showed significant increase ($p < 0.001$) in the mean values of protein than control group. The mean values of protein in the study drug group showed non significant decrease than the induced group and showed significant increase ($p < 0.001$) than the control groups. [Table 6.33].

Table 6.33: Effect of CKC on biochemical parameters of male rats in spermatogenic study			
Groups	**Total Cholesterol (mg/dl)**	**Total Protein (mg /dl)**	**Glycogen (mg/gm)**
Normal Control [I]	102.25 ± 0.3547	5.7±0.0856	13.617±.0872
Induced Group [II]	120.4 ±0.3983 a***	6.29±0.0545 a***	10.34±.07668 a***
Treatment Group[III]	99.36 ±0.4616 b/c***	5.72±0.0475 b ; c***	14.56±.11450 b/c***
Values are expressed as mean ±S.E.M, n=6, a- Group I vs Group II, b-Group II vs. Group III, c - Group I vs. Group III, ***P < 0.001			

Serum testosterone (TT)

The serum TT levels of the ethanol induced group had showed significant ($p < 0.001$) decline. The rats of the treatment group showed significant ($p < 0.001$) recovery in the testosterone level from the induced group. [Table 6.34]

Table 6.34: Effect of CKC on Serum Testosterone level (ng / DL)	
Normal Control -Group I	75.38± 1.2316
Ethanol Induced-Group II	34.40±5.2305 a***
Treatment - Group III	63.30±5.62645 b*** & c
Values are expressed as mean ±S.E.M, n=6, a- Group I vs Group II, b-Group II vs. Group III, c - Group I vs. Group III, ***P < 0.001.	

Effect on sperm indices

Rats exposed to ethanol (25%) group showed significant decrease ($P<0.001$) in the sperm count, motility, normal forms of sperm and viabilty and significant increase ($P<0.001$) in abnormal forms of sperm when compared to that of the control group. Treatment group showed significant increase ($P<0.001$) in sperm count, motility, viability, normal form of sperm and significant decrease ($P<0.001$) in abnormal form of sperm when compared to that of the ethanol induced group. [Table 6.35]

Table 6.35: Effect of CKC treatment on Sperm parameters of rats					
Groups	**Count (million/ml)**	**Motility (%)**	**Morphology (%)**		**Viability (%)**
			Normal	Abnormal	
Normal Control [I]	103.73±1.19	76.31±1.93	86.22±2.45	13.77±2.45	67.27± 1.84
Induced Group [II]	57.32±.58 a***	20.86±1.89 a***	25.06±2.96 a***	74.93±2.96 a***	44.44±1.96 a***
Treatment Group[III]	90.85±5.796 b*** & c	60.28±4.761 b*** & c*	88 ±.8025 ***b & c	11.99±.802 ***b & c	63.02±2.23 b*** & c
Values are expressed as mean ±S.E.M, n=6, a- Group I vs Group II, b-Group II vs. Group III, c - Group I vs. Group III, *P<0.05 , ***P < 0.001.					

Histopathological Examination:

Histocytology of testicular tissue of the control group animals (Group I) showed well differentiated germ cells with respect to spermatogonia which also includes the spermatid and sperm. It was observed that the presence of mature somatic cells projects the perfect histomorphology of the testicular cells in this group. Normal sertoli cell was aligned properly on the basement membrane with oval dome shaped nucleus which showed the normal morphology of the seminiferous tubule. (fig 6.34 A,B; fig 6.35)

Microscopic observation of Group II animals (ethanol) showed decreased number in normal sertoli cells with the irregular cytoarchitecture, further there is evident of decrease in the number of spermatogenic cells into the lumen of seminiferous tubule. The Primary spermatocytes of the sample belonging to group II showed condensed chromatin similarly the number of leptotenes and zygotenes are very minimal in this group. Arrangement of connective tissue surrounds many very minimal numbers of polygonal cells. (fig 6.36 A, B). Size and shape of the sperm appears abnormal in the group treated with alcohol. (fig 6.37)

Histological examination of rat testis belonging to Group III (*CKC*) stained by hematoxylin and eosin showed many seminiferous tubules encircled by prominent membrane. Leptotene and zygotene spermatocytes appeared rich in number. Primary spermatocytes with large centered nucleus and dense chromatin were observed. Seminiferous tubules showed plenty of sertoli cells with normal histology and also increased spermatogenic cells into the lumen of seminiferous tubule. The seminiferous tubules showed normal spermatogenesis and spermiation.Sertoli cells with apparent triangular nucleus were observed in the group supplemented with test drug. And also proper distribution of collagen fibers along with the seminiferous tubule was observed. Tubules appeared to be uniform in size and shape. Specimen belonging to the group III showed normal flat basement membrane. Absolute interstitial layer was observed. Animals treated with test drug showed normal morphology of cauda epithelial layer and also numerous number of spermatid cells were observed. Presence of epididymal lobule was normal. This group showed normal flat basement membrane. Size and shape of the Spermatozoa were observed to be normal.

Histometric study

Diameter of Tubules

Histogram of seminiferous tubules of the control group animals were 206.6, 205.9 and 203.8 μm as showed by reading on the micrometer scale. Ethanol treated animals showed shrunken pattern evident by decrease in the width (103.6; 157.7; 122.4 μm) and in treatment group it is recovered (183.9; 192.7; 210.7 μm) from the induced group. (fig 6.38)

Diameter of Testis

Measurement of thickness of testis in the control group animals were 183.7 x 128.6, 186.6 x 131.8 and 201.6 x 107.2 μm as showed by reading on micrometer scale. Ethanol induced group animals showed shrunken values (180.0 x 126.7, 141.8 x 142.0, 155.0 x 126.7 μm) and in the treatment group it was recovered (174.3 x 137.7, 188.4x137.0, 238.8 X 134.1 and 208.6x125.4 μm) from the ethanol induced group.(fig 6.39)

Clinical study

Pilot study

Haematological parameters

The mean value of Hb (15.79; 15.9), RBC (5.26; 5.24), T.WBC (7900.0 ; 7940.0) and platelets (2.74; 2.76) were statistically non-significant (p >0.05) AT (after treatment) and AFU (after follow up). The mean value of ESR (5.2) showed significant decrease (P<0.01**)** AT (within normal range) and showed non significant changes (4.80 ; p > 0.05) AFU.[Table 6.36 A, B]

Table 6.36A: Effect of CKC on haematological parameters in pilot study (AT)

Haematology	Mean		Mean diff	S.D	S.E	t	p
	BT	AT					
HB	15.72	15.9	0.18	0.178	0.080	2.25	p > 0.05
RBC	5.28	5.26	0.02	0.258	0.115	0.17	
T.WBC	7660.0	7900.0	240.0	250.9	112.5	2.13	
Platelets	2.68	2.74	0.06	0.151	0.67	0.88	
ESR	6.8	5.2	1.6	0.894	0.4	4.0	P<0.01**
Values are expressed as mean ±S.E.M, n=5, p-values :Significance levels: **P<0.01							

Table 6.36 B: Effect of CKC on haematological parameters in pilot study (AFU)

Haematology	Mean		Mean diff	S.D	S.E	t	p
	BT	AFU					
HB	15.72	15.9	0.18	0.148	0.0663	2.714	p > 0.05
RBC	5.28	5.24	0.04	0.151	0.0678	0.590	
ESR	6.8	4.80	2.0	2.0	0.894	2.236	
T.WBC	7660.0	7940.0	280.0	258.844	115.758	2.419	
Platelets	2.68	2.76	0.08	0.30331	0.1356	0.590	
Values are expressed as mean ±S.E.M, n=5, p-values : non Significant: p > 0.05							

Biochemical parameters

- The mean value of blood sugar (102.2; 104.0), HDL (38.6; 38.2), urea (22.0; 22.0), creatinine (0.82 0.84), total bilirubin (0.90, 0.88), total protein (6.16, 6.32) showed non significant changes both AT and AFU.
- Serum cholesterol showed significant decrease AT (153.4; p < 0.001) and AFU (153.0; p < 0.05).

Table 6.37A: Effect of CKC on biochemical parameters in pilot study (AT)

Biochemical	Mean		Mean diff	S.D	S.E	t	p
	BT	AT					
R.Sugar	105.6	102.2	3.4	3.286	1.47	2.313	p > 0.05
S.Cholesterol	158.4	153.4	5.0	1.414	0.632	7.906	P<0.001***
HDL	37.0	38.6	1.60	2.702	1.208	1.324	p > 0.05
LDL	90.8	85.2	5.60	2.510	1.122	4.989	P<0.001***
VLDL	45.8	40.4	5.40	0.548	0.245	22.045	P<0.001***
TGL	177.2	162.4	14.8	9.445	4.224	3.504	p < 0.05*
B.Urea	22.6	22.0	0.60	3.130	1.40	0.429	p > 0.05
S.Creatinine	0.80	0.82	0.02	0.04	0.02	1.0	p > 0.05
Uric acid	4.08	3.120	0.96	0.250	0.112	8.552	P<0.001***
T.Bilirubin	0.90	0.90	0.00	0.14	0.06	0.00	p > 0.05
T.Protein	6.76	6.16	0.6	0.651	0.2915	2.058	p > 0.05
AST	31.4	23.6	7.80	1.924	0.860	9.067	P<0.001***
ALT	41.4	35.0	6.40	4.775	2.135	2.997	p < 0.05*
SAP	165.6	160.4	5.20	1.095	0.490	10.614	P<0.001***
Values are expressed as mean ±S.E.M, n=5, p-values : Significance levels: *p < 0.05, ***P<0.001							

Table 6.37 B: Effect of CKC on biochemical parameters in pilot study (AFU)

Biochemical	Mean		Mean diff	S.D	S.E	t	p
	BT	AFU					
R.Sugar	105.6	104.0	1.60	3.362	1.503	1.064	p > 0.05
S.Cholesterol	158.4	153.0	5.40	4.09	1.833	2.946	p < 0.05*
HDL	37.0	38.2	1.20	4.382	1.960	0.612	p > 0.05
LDL	90.8	85.6	5.20	5.119	2.289	2.272	p > 0.05
VLDL	45.8	39.6	6.2	1.643	0.735	8.437	p < 0.001***
TGL	177.2	165.4	11.80	7.918	3.541	3.332	p > 0.05
B.Urea	22.6	22.0	0.60	2.793	1.249	0.480	p > 0.05
S.Creatinine	0.80	0.84	0.04	0.089	0.04	1.00	p > 0.05
Uric acid	4.08	3.40	0.68	0.248	0.1113	6.107	p < 0.001***
T.Bilirubin	0.90	0.88	0.02	0.1304	0.0583	0.343	p > 0.05
T.Protein	6.76	6.32	0.440	0.7369	0.3295	1.335	p > 0.05
AST	31.4	26.8	4.60	1.57	0.678	6.782	p < 0.001***
ALT	41.4	36.6	4.80	3.421	1.530	3.138	p < 0.05*
SAP	165.6	161.0	4.60	2.074	0.927	4.960	p < 0.001***
Values are expressed as mean ±S.E.M, n=5, Significance levels: *p < 0.05, ***p < 0.001							

- LDL showed significant decrease (85.2, P<0.001) AT and non significant changes (85.6, P<0.05) AFU. TGL showed significant decrease (162.4, $p < 0.001$) AT and showed non significant changes (165.4, $p > 0.05$) AFU.
- VLDL (40.4, 39.6), uric acid (3.12, 3.40), AST (23.6, 26.8), SAP (160.4, 161.0) showed significant decrease ($p < 0.001$) AT and AFU.
- ALT (41.4, 36.6) showed significant decrease ($p < 0.05$) AT and AFU. All were within normal range.

Semen Parameters

The trial drug provided significant increase (P < 0.001) in the sperm count (41.6, 36.0) progressive motility (39.8, 36.2), total motility (51.2, 50.0) and normal form of sperm (53.8, 51.8) both AT and AFU. Non significant changes ($p > 0.05$) was observed in the volume (1.8, 1.60) and the liquification time (26.0, 26.0) both AT and AFU.

Table 6.38 A: Effect of CKC on semen parameters in pilot study (AT)

Semen Analysis	Mean		Mean diff	S.D	S.E	t	p
	BT	AT					
Count	8.6	41.6	33.0	12.708	5.683	5.806	P<0.001***
Pr.Motility	9.6	39.8	30.2	3.834	1.715	17.61	
T.Motility	23.2	51.2	28.0	7.176	3.209	8.724	
Morphology	34.2	53.8	19.6	4.827	2.159	9.080	
Volume	1.3	1.8	0.50	0.50	0.223	2.236	p > 0.05
Liquification	27.0	26.0	1.0	4.183	1.871	0.535	
Values are expressed as mean ±S.E.M, n=5, p-values :Significance levels: ***P<0.001							

Table 6.38 B: Effect of CKC on semen parameters in pilot study (AFU)

Semen Analysis	Mean		Mean diff	S.D	S.E	t	p
	BT	AFU					
Count	8.6	36.0	27.4	12.708	5.683	5.806	P<0.001***
Pr.Motility	9.6	36.2	26.60	2.608	1.166	22.80	
T.Motility	23.2	50.0	26.8	12.43	5.562	4.818	
Morphology	34.2	51.8	17.60	3.847	1.720	10.230	
Volume	1.3	1.60	0.30	0.908	0.4062	0.739	p > 0.05
Liquification	27.0	26.0	1.0	6.519	2.915	0.343	
Values are expressed as mean ±S.E.M, n=5, p-values :Significance levels: ***P<0.001							

Harmone Parameters

The mean value of testosterone (5.70, 5.55) and LH (6.46, 6.29) showed significant increase (P <0.001) AT and AFU and FSH showed significant increase (7.74, p < 0.01) AT and (7.40, p < 0.05) AFU.

Table 6.39 A: Effect of CKC on harmone parameters in pilot study (AT)

Harmone	Mean		Mean diff	S.D	S.E	t	p
	BT	AT					
Testosterone	4.16	5.70	1.536	0.172	0.077	19.869	P<0.001***
FSH	6.63	7.74	1.110	0.544	0.243	4.556	p < 0.01**
LH	5.612	6.46	0.854	0.119	0.053	16.008	P<0.001***
Values are expressed as mean ±S.E.M, n=5, p-values : Significance levels: **p < 0.01, ***P<0.001							

Table 6.39 B: Effect of CKC on harmone parameters in pilot study (AFU)

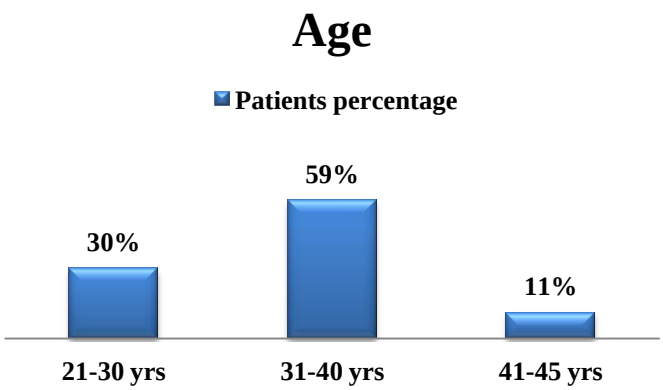
Harmone	Mean		Mean diff	S.D	S.E	t	p
	BT	AT					
Testosterone	4.16	5.558	1.392	0.2394	0.107	13.0	P<0.001***
FSH	6.63	7.406	0.776	0.5539	0.247	3.132	p < 0.05*
LH	5.612	6.298	0.686	0.1234	0.055	12.43	P<0.001***
Values are expressed as mean ±S.E.M, n=5, p-values :Significance levels: *p < 0.05, ***P<0.001							

Main Clinical Trial

I] Observation based on patients general status

1) Age (n-37)

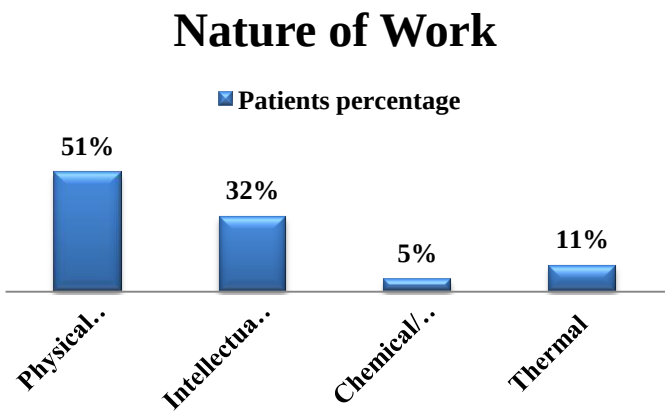
Fig 6.40A: Observation of patients based on age



It was observed that the maximum of 22 (59%) patients were found between the age group of 31 to 40 years, 11 (30 %) patients were between 21-30 years and 4 (11 %) patients were between 41-45 years.

2) Nature of work (n-37)

Fig 6.40 B: Observation of patients based on Nature of Work

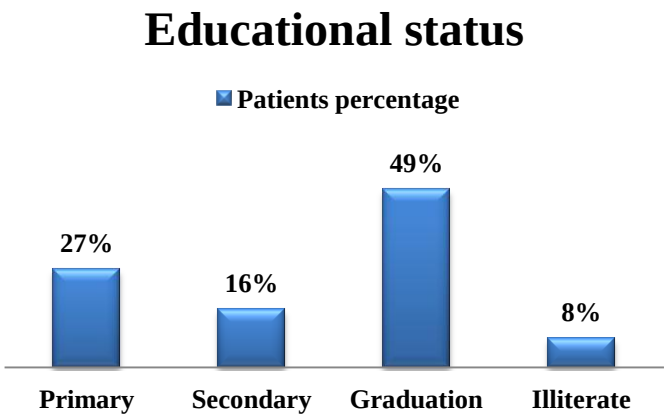


Majority of the patients 19 (51 %) were doing physical exertional work, 12 (32 %) patients were doing intellectual work, 02 (5 %) patients were doing chemical/radiation work and 04 (11%) patients comes under thermal nature of work.

3) Educational status (n-37)

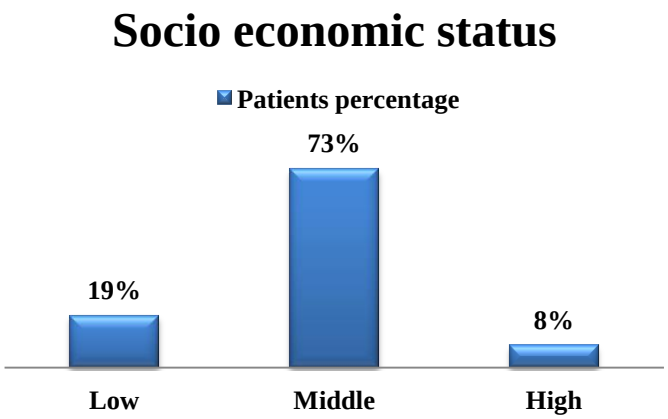
10 (27%) patients had primary education, 6 (16%) had secondary education,18 (49%) had graduation and 3 (8%) were illliterate.

Fig 6.40 C: Observation of patients based on Educational Staus



4) Socio economic status (n-37)

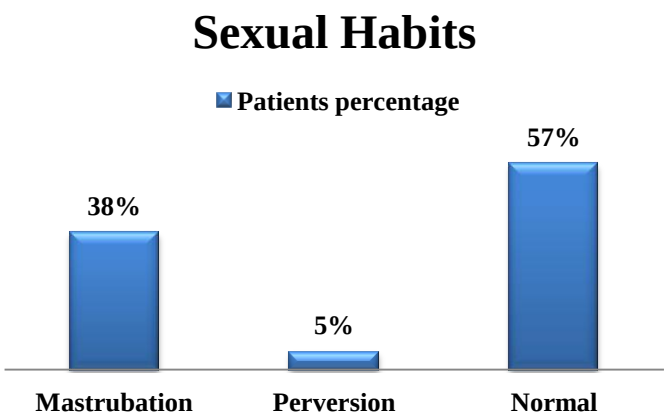
Fig 6.40 D: Observation of patients based on Socio Economic Staus



27 (73%) patients comes under middle economic status and 7 (19%) patients of low economic status and 3 (8%) patients of high economic status.

5) Sexual habit (n-37)

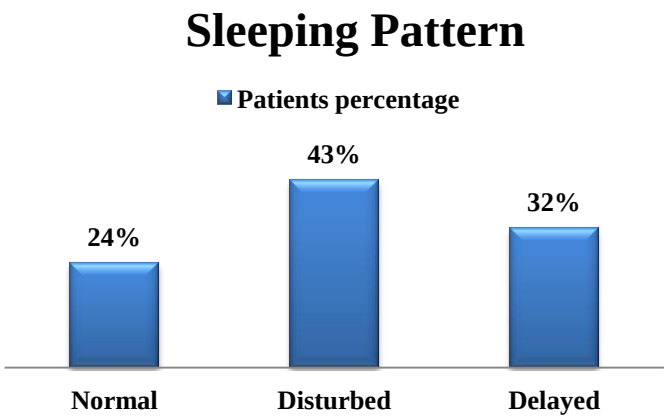
Fig 6.40 E: Observation of patients based on Sexual habits



21 (57%) patients comes under normal, 2 (5%) patients comes under perversion and 14 (38%) patients had the history of masturbation before marriage.

6) Sleeping pattern (n-37)

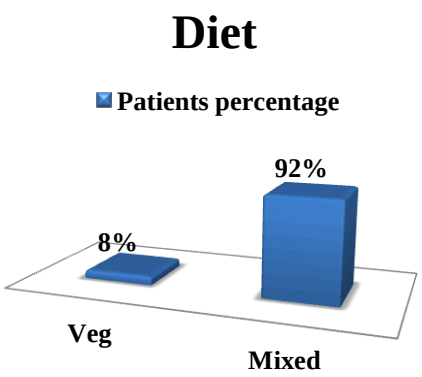
Fig 6.40 F: Observation of patients based on Sleeping Pattern



Only 9 (24%) patients had sound sleep, 16 (43%) had disturbed sleep and 12 (32%) patients were having delayed sleep

7) Diet (n-37)

Fig 6.40 G: Observation of patients based on Diet

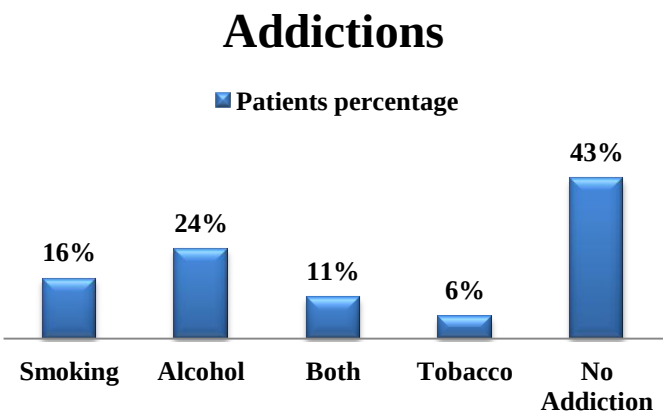


Majority of 34 patients (92%) were of mixed type and remaining 3 (8%) patients were vegetarian.

8) Addiction (n-37)

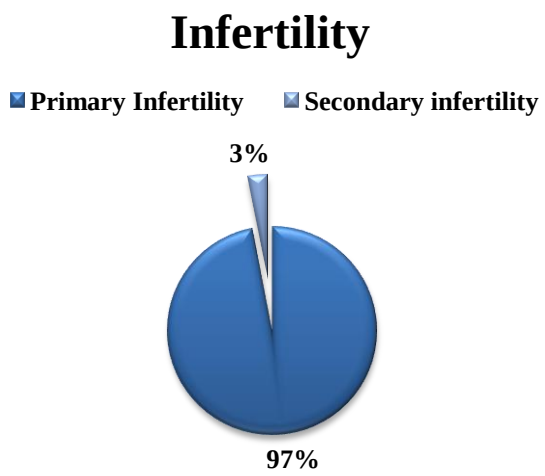
6 patients (16%) were addicted to smoking, 9 (24 %) patients to alcohol, 2 patients (6 %) to tobacco, 4 patients (11 %) both to smoking and alcohol and 16 (43%) patients were having no addiction

Fig 6.40 H: Observation of patients based on Addictions



9) Infertility (n-37)

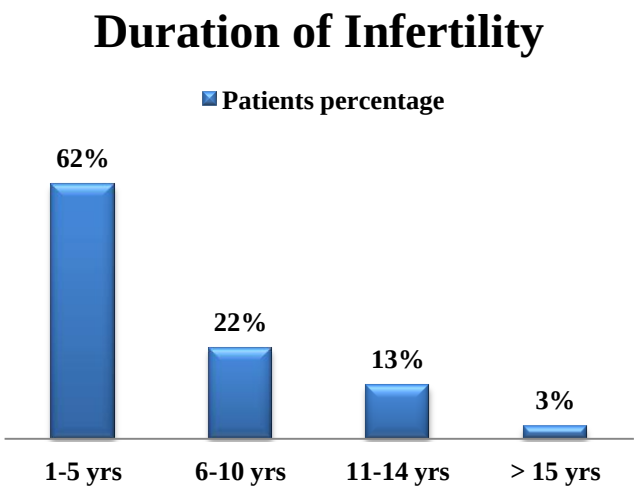
Fig 6.40 I: Observation of patients based on Infertility



36 (97%) patients were presented with primary infertility and 1 (3%) patient complained of secondary infertility.

10] Duration of infertility (n-37)

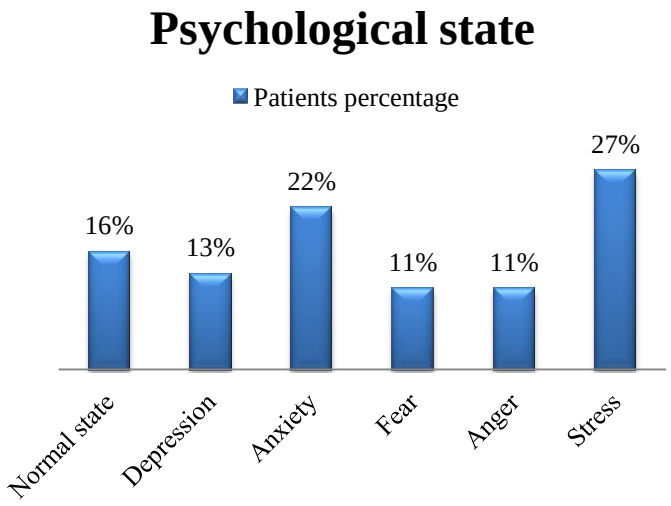
Fig 6.40 J: Observation of patients based on duration of infertility



Highest proportion of duration of infertility was observed between 1-5 years in 23 (62%) patients, 8(22%) patients showed duration between 6-10 years, 5(13%) patients showed between 11-14yrs and in 1 (3%) patient the duration was found above 15 years.

11) Psychological state (n-37)

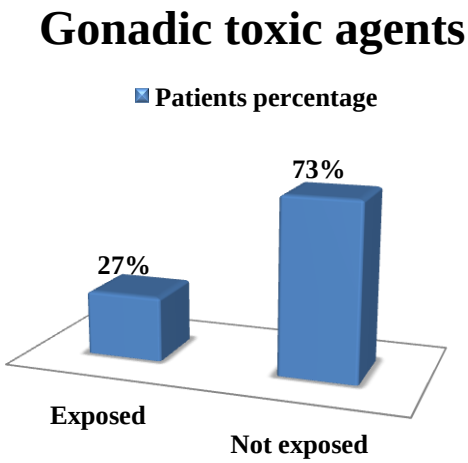
Fig 6.40 K: Observation of patients based on Psychological State



6 (16%) patients had normal psychological status, 5 (13%) patients were in depression, 8 (22%) patients were in anxious state, 4 (11%) patients were in fear and anger state each and 10 (27%) were under stress

12) Gonadotoxic agents (n-37)

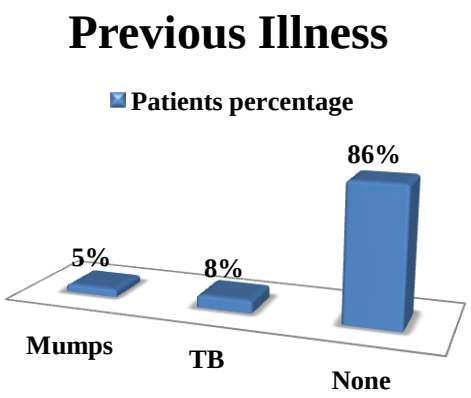
Fig 6.40 L: Observation of patients based on Gonadic toxic agents



Maximum of 27 (73%) patients had no history of exposure to gonadotoxic agents and 10 (27 %) patients had history of exposure to gonadotoxic agents.

13) Previous illness (n-37)

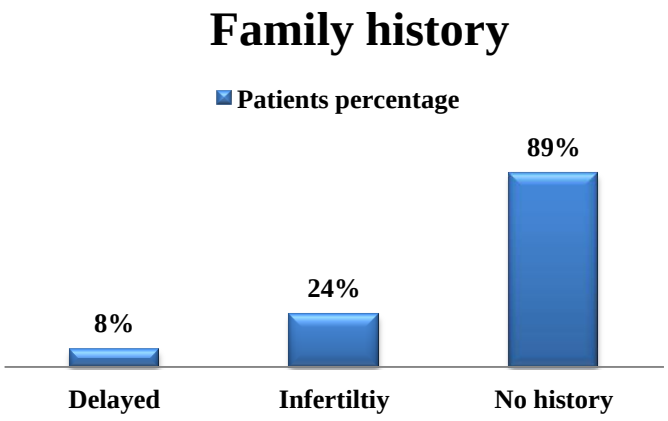
Fig 6.40 M: Observation of patients based on Previous Illness



2 (5%) patients had the history of mumps, 3 (8%) patients had TB and maximum of 33 (86%) patients had no exposure to previous illness.

14) Family history (n-37)

Fig 6.40 N: Observation of patients based on Family History

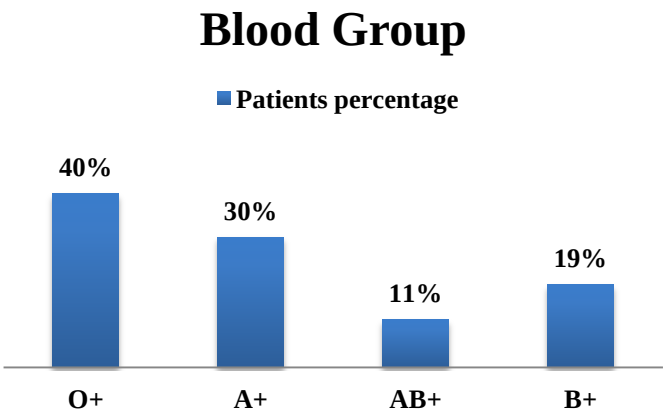


Only 3 (8%) of the patients had the family history of delayed conception in mother or siblings, 1 (3%) patient had history of infertility in siblings and 33 (89%) patients had no family history

15) Blood group (n-37)

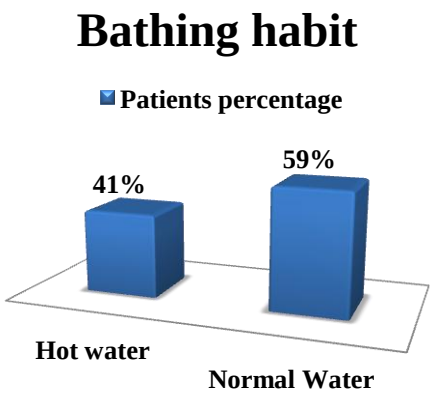
Maximum of 15 (40%) patients reported O+ group, 11 (30%) patients reported A+ group, 4 (11%) patients reported AB+ group and 7 (19%) patients reported B+ group.

Fig 6.40 O: Observation of patients based on Blood Group



16) Bathing habit (n-37)

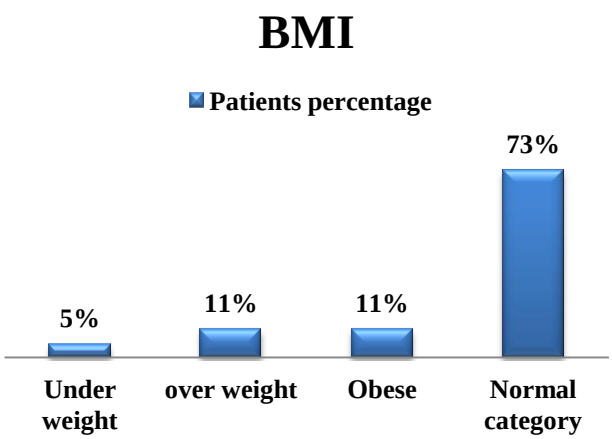
Fig 6.40 P: Observation of patients based on bathing habit



15 (41%) patients were having the habit of hot water bath and 22 (59%) patients were having the habit of normal water bath.

17) BMI (n-37)

Fig 6.40 Q: Observation of patients based on BMI



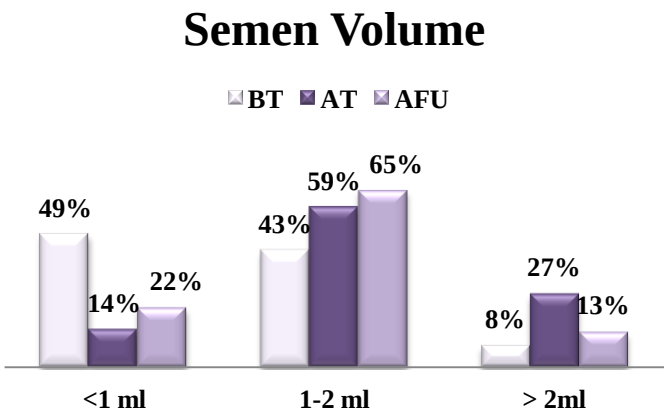
BMI signifies 2 (5 %) patients were underweight, 4 (11%) patients were over weight, 4 (11%) patients were obese and 27 (73%) comes under normal category.

II] Observation based on Laboratory findings

Semen Parameters

1) Semen volume (n-37)

Fig 6.41A: Observation of patients based on Semen Volume

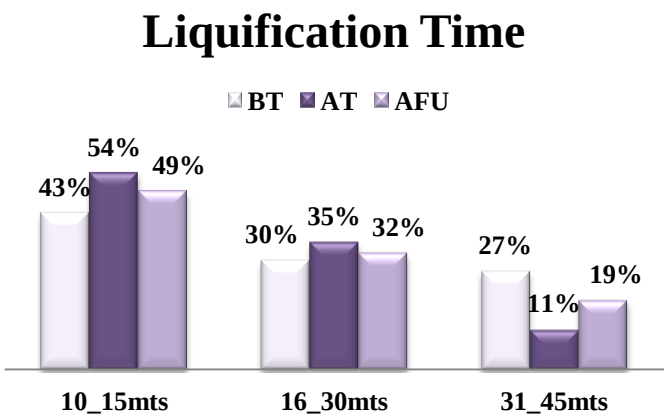


BT 18 (49%) patients were reported with semen volume below 1ml; 16 (43%) patients reported semen volume of 1-2 ml and rest 3 (8%) patients reported semen volume of more than 2 ml. AT 5 (14%) patients reported semen volume below 1ml, 22 (59%) patients showed improvement in semen volume between 1 –2 ml and 10 (27%) patients showed improved volume more than 2ml. AFU 8 (22%) patients reported volume below 1ml, 24 (65

%) patients reported volume between 1-2ml and 5 (13%) patients reported volume of above 2ml.

2) Liquification time (n-37)

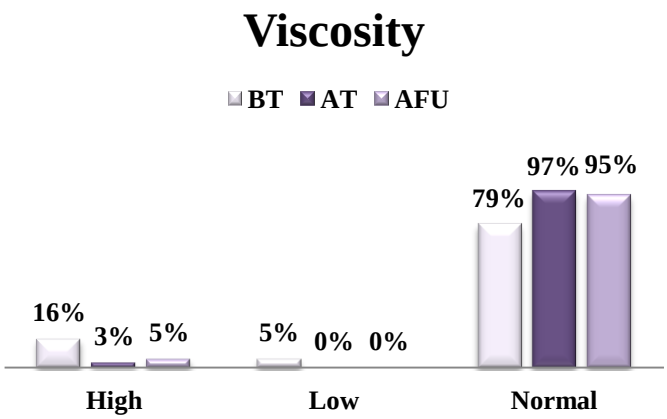
Fig 6.41 B: Observation of patients based on liquification time



Liquification time of the patients BT was 16 (43%) in 10_15 mts, 11 (30 %) in 16_30mts, 10 (27%) in 31_45mts and AT with the proportion of 20 (54%) patients in 10_15mts, 13 (35%) patients in 16_30mts, 4 (11%) patients in 31_45mts and AFU showed the proportion of 18 (49%) patients in 10_15mts, 12 (32%) patients in 16_30mts and 7 (22%) patients in 31_45 mts.

3) Semen Viscosity (n-37)

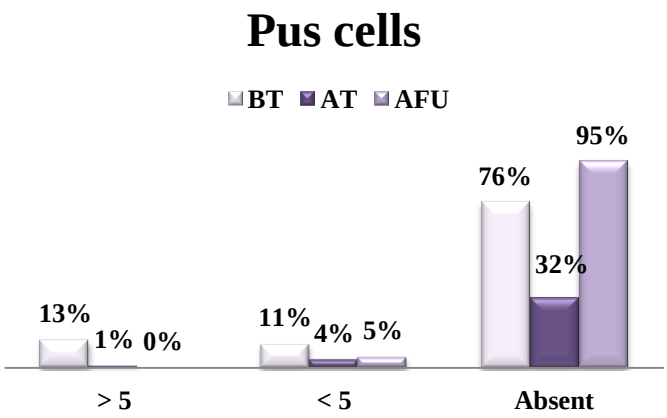
Fig 6.41 C: Observation of patients based on Viscosity



BT 6 (16 %) patients showed high viscous semen, 2 (5 %) patients of low visous semen and AT the values decreased to 1(3%) patient in high visous semen, no patients (0%) in low viscous semen and AFU the values were 2 (5%) patients in high viscous semen and no patients (0 %) in low visous semen**.**

4) Puscells (n-37)

Fig 6.41 D: Observation of patients based on pus cells

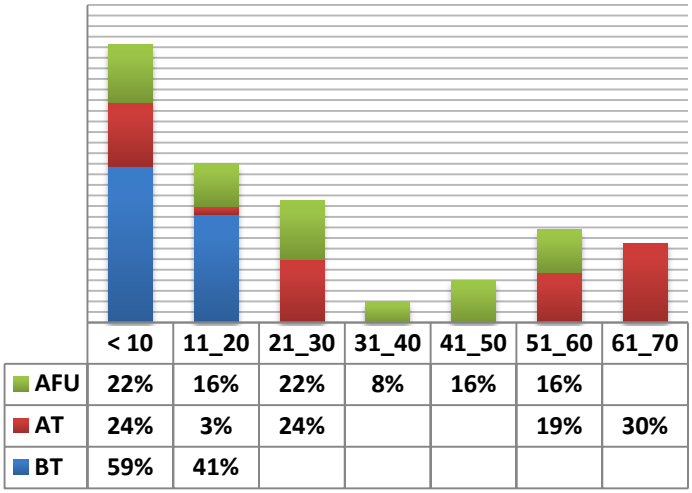


BT 5 (13%) patients showed pus cells of more than value of 5, 4 (11%) patients showed less than 5 and AT the values decreased to 1 (3%) in more than 5, 4 (11%) patients in less than 5 and AFU the values were no patients (0%) in more than 5 and 2 (5%) patients in less than 5.

5) Sperm count (n-37)

Out of 37 patients, BT 22 (59%) patients were having sperm count below 10 millions/ml; 15 (41%) patients were having sperm count 11-20 millions/ml, AT 9 (24%) patients were having count below 10 millions/ml, 1 (3%) patient showed improvement up to 11-20 millions/ml; 9(24%) patients were improved in sperm count of 21-30 millions/ml; 7 (19%) patients were improved up to sperm count of 51 – 60 millions/ml, 11(30%) patients were improved up to sperm count of 61- 70 millions/ml; and AFU 8 (22%) patients reported count below 10 millions/ml, 6 (16) patients reported up to 11- 20 millions/ml; 8 (22%) patients reported sperm count up to 21-30 millions/ml;3(8%) patients reported count up to 31-40millions /ml, 6(16%) patients reported count of 41-50 millions/ml and 6 (16%) patients reported count up to 51-60 millions /ml.

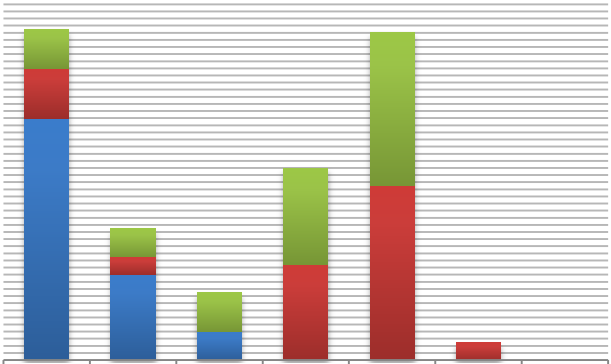
Fig 6.41 E: Observation based on Sperm count



	< 10	11_20	21_30	31_40	41_50	51_60	61_70
AFU	22%	16%	22%	8%	16%	16%	
AT	24%	3%	24%			19%	30%
BT	59%	41%					

6) Progressive Motility (n-37)

Fig 6.41 F: Observation of patients based on Progressive Motility



	0_10	11_20	21_30	31_40	41_50	51_60	
AFU	11%	8%	11%	27%	43%		
AT	14%	5%		27%	49%	5%	
BT	68%	24%	8%				

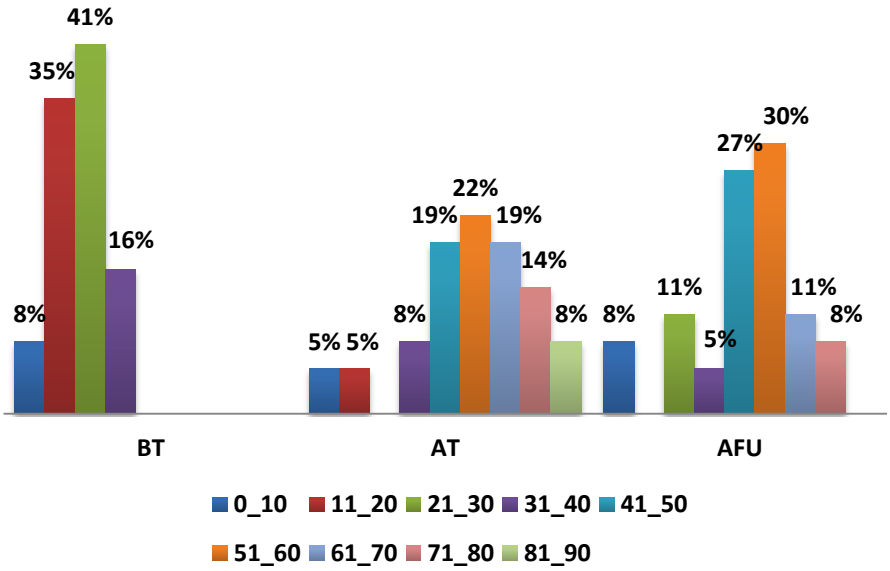
BT out of 37 patients 25 (68%) patients reported progressive motility of 0-10%; 9(24%) patients were having progressive motility of 11-20%; 3 (8%) patients were having progressive motility of 21-30%; AT 5 (14%) patients were having progressive motility of 0-10 %, 2 (5 %) patients improved up to 11-20%; 10 (27%) patients improved up to 31-40%; 18 (49 %) patients improved up to 41-50%; 2 (5%) patients improved up to 51-60% and AFU 4(11%) patients reported progressive motiliy of 0-10%, 3(8%) patients reported up to 11-

20%, 4(11%) patients reported up to 21-30%, 10(27%) patients reported up to 31-40%, 16(43%)patients reported up to 41-50%.

7) Total Motility (n-37)

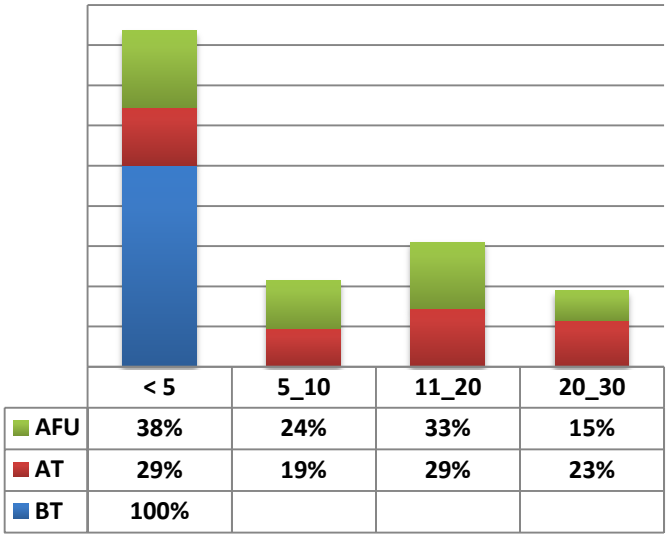
BT out of 37 patients, 3 (8%) patients were reported total motility of 0-10%; 13(35%) patients were having total motility of 11-20%; 15 (41%) patients were having total motility of 21-30%; 6(16%) were having total motility of 31-40%. AT 2 (5%) patients were having progressive motility of 0-10 %, 2 (5%) patients improved up to 11-20%; 3(8%) patients improved up to 31-40%; 7 (19%) patients improved up to 41-50%; 8 (22%) patients improved up to 51-60%,7(19%) patients improved up to 61-70%, 5(14%) patients improved up to 71-80%, 3(8%) patients improved up to 81-90%. AFU 3 (8%) patients reported progressive motiliy of 0-10%, 4(11%) patients reported up to 21-30%, 2(5%) patients improved up to 31-40%, 10(27%) patients reported up to 41-50%, 11(30%) patients reported up to 51-60%, 4(11%)patients reported up to 61-70%, 3(8%) patients reported 71-80% of total motility.

Fig 6.41 G: Observation of patients based on Total Motility



8) Normal sperms (n-21)

Fig 6.41 H: Observation of patients based on Normal form of Sperm



	< 5	5_10	11_20	20_30
AFU	38%	24%	33%	15%
AT	29%	19%	29%	23%
BT	100%			

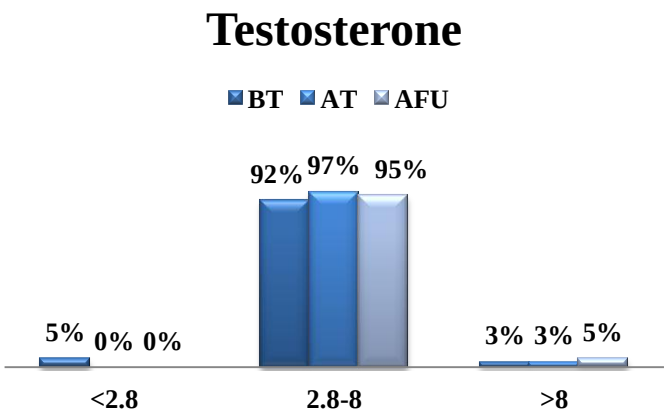
BT 21 patients were having less than normal form of sperm. AT out of 21 patients, 6 (29%) patients were having 0-5 % normal form of sperm, 4 (19%) patients improved up to 5-10%; 6(29%) patients improved up to 11-20%; 5 (23%) patients improved up to 20-30%; AFU 8 (38%) patients reported normal form of sperm of 0-5%, 5 (24%) patients reported up to 5-10%, 7 (33%) patients reported up to 11-20%, 1(5%) patients reported up to 20-30% of normal form of sperm.

Harmone parameters

9) Testosterone (n-37)

BT 2 (5%) patients were having testosterone below 2.8 ng/ml, 34 (92%) were having range between 2.8 to 8 ng/ml, 1(3%) patients were having above 8 ng/ml. AT 36(97%) patients were improved to the range between 2.8 to 8ng/ml and 1 (3%) patient was having above 8ng/ml value. AFU 35(95%) patients were reported to have 2.8 to 8ng/ml testosterone and 2 (5%) patients were reported to have above 8ng/ml of testosterone.

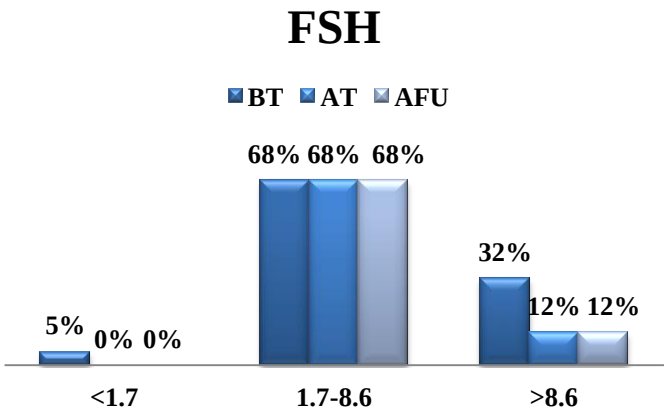
Fig 6.42A: Observation of patients based on Testosterone



10) FSH (n-37

BT 25(68%) patients were having FSH in the range between 1.7 to 8.6 mIu/ml, 12 (32%) patients were above 8.6 mIu/ml, AT and AFU same proportion was reported.

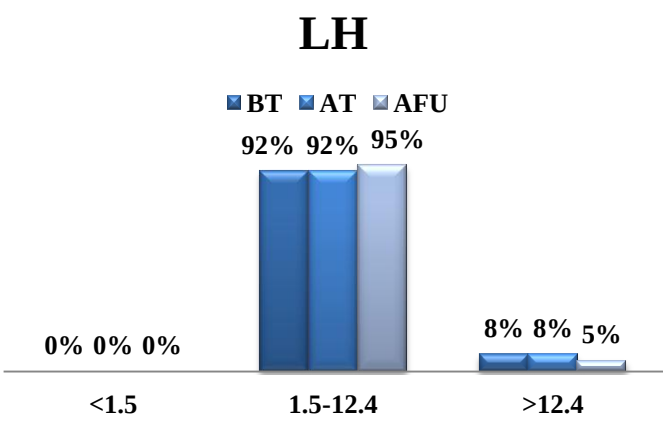
Fig 6.42 B: Observation of patients based on FSH



11) LH (n-37)

BT 34 (92%) patients were having LH in the range between 1.5-12.4mIu/ml, 3(8%) patients were having LH above 12.4 mIu/ml. AT same proportion was reported. AFU 35(95%) patients reported the range between 1.5 to 12.4mIu/ml and 2 (5%) patients reported to have above 12.4mIu /ml.

Fig 6.42 C: Observation of patients based on LH



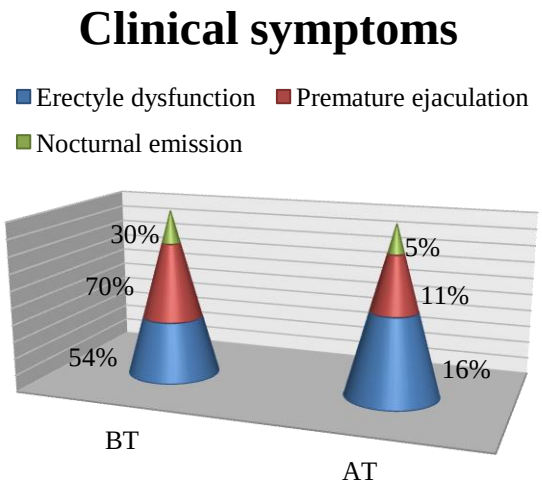
12) Urine routine:

Albumin, Sugar, bile salts, bile pigments, Urobilinogen, Acetone, occult blood all the parameters were found to be absent BT, AT and AFU.

III] Observation based on Clinical symptoms (n-37)

BT 20 (54%) patients with erectyle dysfunction, 26 (70%) patients with premature ejaculation and 11(30%) patients with nocturnal emission were reported and AT 6 (16%) patients with erectyle dysfunction, 4 (11%) patients with premature ejaculation and 2 (5%) patients with nocturnal emission were reported.

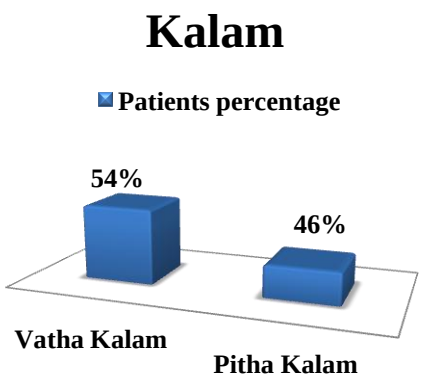
Fig 6.43: Observation of patients based on Clinical Symptoms



IV] Observation of patients based on *Siddha* clinical parameters

1) *Kaalam* [Patients age] (n-37)

Fig 6.44A: Observation of patients based on *Kaalam*

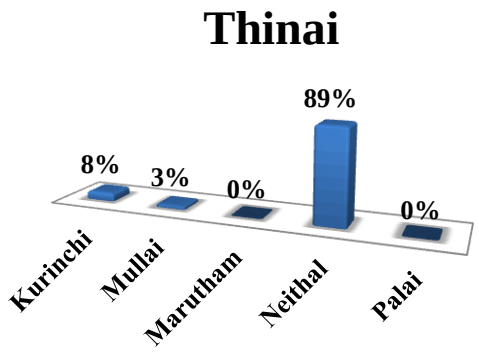


Among 37 cases, 54% of cases came under vatha kaalam the age group between 1-33 years and 46 % of cases came under pitha kaalam the group between 34-66 years.

2) *Thinai* (Habitat) (n-37)

Majority of 15 (89 %) patients were reported from neithal thinai (costal tract), 3 (8 %) patients reported from kurinchi thinai [hilly tract] and 1 (3%) patient reported from mullai thinai [sylvan tract].

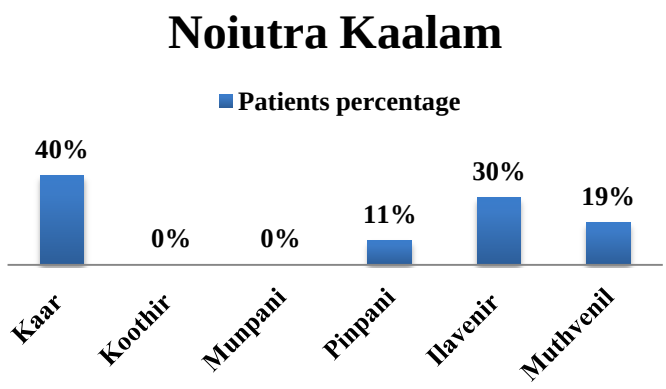
Fig 6.44 B: Observation of patients based on *Thinai*



3) *Noi utra kaalam* (Season) (n-37)

Among 37 cases, 15 (40%) patients reported during Kaar kaalam[rainy season - mid august to mid october], 4 (11%) patients reported during pinpanikalam [early winter-mid february tomid april], 11 (30%) patients reported during Ilavenirkaalam [early summer-mid april to mid june], 7 (19%) patients reported during Muthuveneerkaalam [late summer-mid june to mid august] and no patients (0%) reported during Koothirkaalam [late rainy season-mid october to mid december] munpanikalam [latter winter-mid december to mid february].

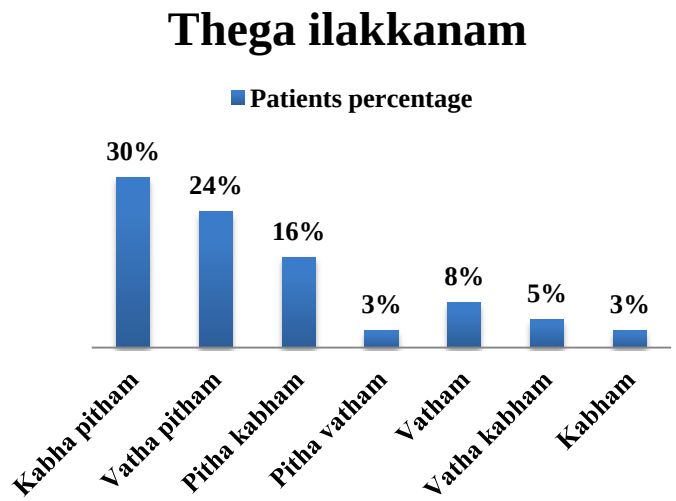
Fig 6.44 C: Observation of patients based on *Noiutra Kaalam*



4) *Thega ilakkanam* (bio type) (n-37)

Majority of the patients were of kabha pitham biotype with the proportion of 30%, 24% of vathapitham, 16% of pitha kabham, 14 % of pitha vatham, 8% of vatham, 5% of vatha kabham and 3 % of kabham biotype

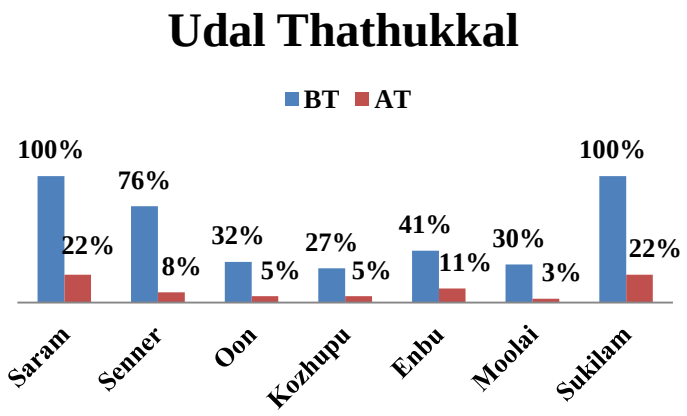
Fig 6.44 E: Observation of patients based on Thega ilakkanam



5) *Udal Thathukkal* (Physical constituents) (n-37)

Before treatment all the (100%) patients had deranged *saaram* (chyle) and *sukkilam* (semen), 76% patients had deranged *senneer* (blood). 32% patients had deranged *oon* (muscle), 27% patients had deranged *kozhuppu* (fat), 41%patients had deranged *enbu* (bone), 30% patients had deranged *moolai* (bone marrow) and after treatment it was reduced to 22% of *saram* and *sukkilam*, 8% of *senneer*, 5% of *oon* and *kozhuppu*, 11% of *enbu*, 3% of *moolai*

Fig 6.44 F: Observation of patients based on Udal Thathukkal

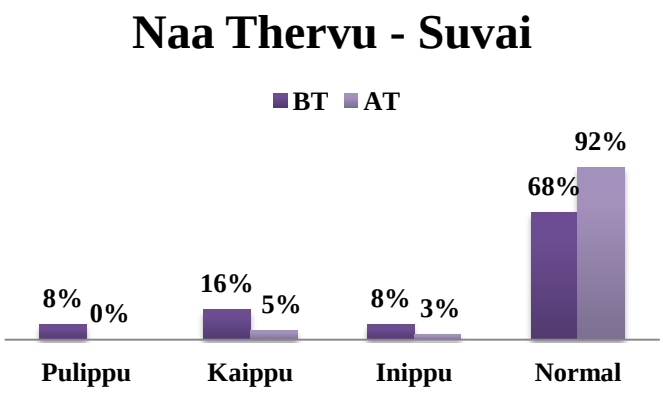


6) *Envagai thervu* (Eight fold examinations) (n-37)

i) Naa Thervu (Tongue examination)

Among 37 cases BT 25 (68%) patients had normal taste in their tongue, 6 (16 %) patients had bitter taste in their in tongue, 3 patients (8%) with sour taste and sweet taste in their tongue, and AT 34 (92%) patients had normal taste, 2 (5%) patients with bitter taste, no patients (0%) with sour taste and 1 (3 %) patient with sweet taste in the tongue.

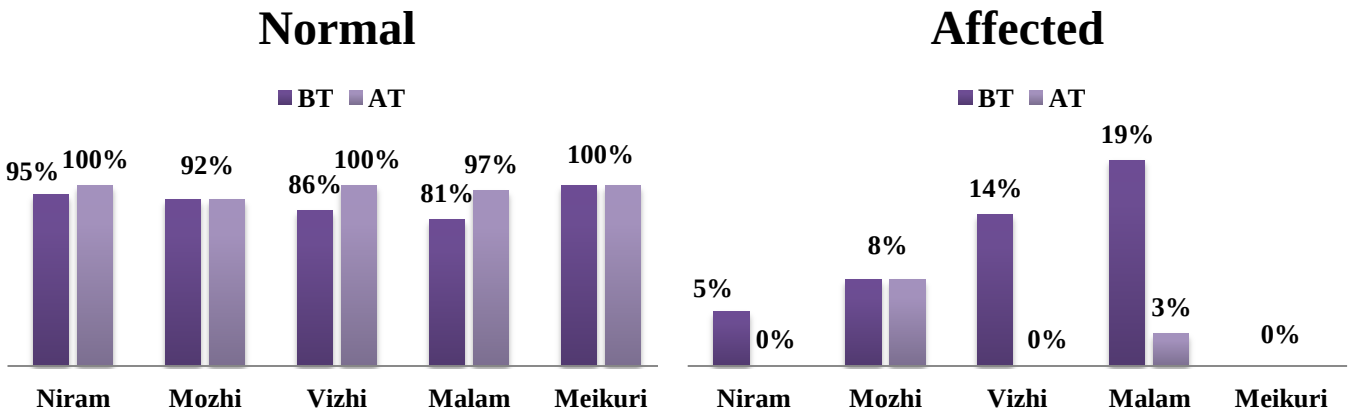
Fig 6.45 A: Observation of patients based on Suvai



ii) Niram [Colour], Mozhi (Voice), Vizhi (Eye), Malam (Faeces), Meikuri (Touch)

Among 37 cases BT 35 (95%) patients had normal skin colour and 2 (5%) with affected skin colour (pallor) and AT all the 37(100%) patients were with normal skin colour. BT 34 (92%) patients with normal voice, 3 (8%) patients with affected voice (Thantha oli) and AT the same proportion was reported. BT 32 (86%) patients had normal eye, 5 (14%) patients with affected eye (pallor, burning sensation) and AT all the patient reported with normal eye. BT 30 (81%) patients faeces were normal, 7 (19%) patients faeces were affected (constipation) and AT 36 (97%) patients faeces were normal, 1 (3%) patient faeces was affected. BT and AT all the 37 (100 %) patients were with normal meikuri (normal body temperature, no tenderness)

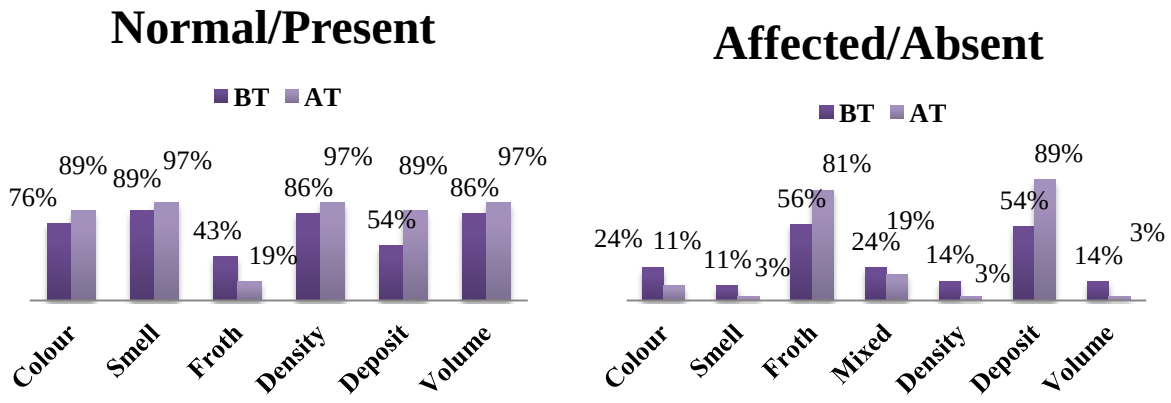
Fig 6.45 B: Observation of patients based on Niram, Mozhi, Vizhi , Malam, Meikuri



***iii)Neerkuri* (Urine examination)**

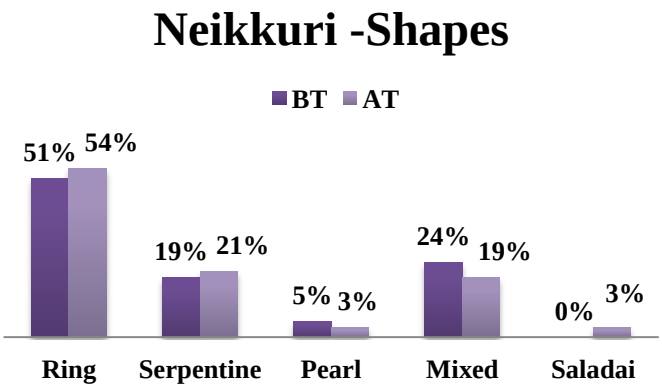
Before treatment 76 % of patients had normal colour, 89% of normal smell, 86 % of normal specific gravity and volume, froth was present in 43 % of patients, deposits was present in 46 % of patients and after treatment patients had 89 % of normal urine colour, 97 % of normal smell, specific gravity and volume, froth was present in 19% of patients, deposits (pus cells) was present in 11% of patients.

Fig 6.45 C: Observation of patients based on *Neerkuri*



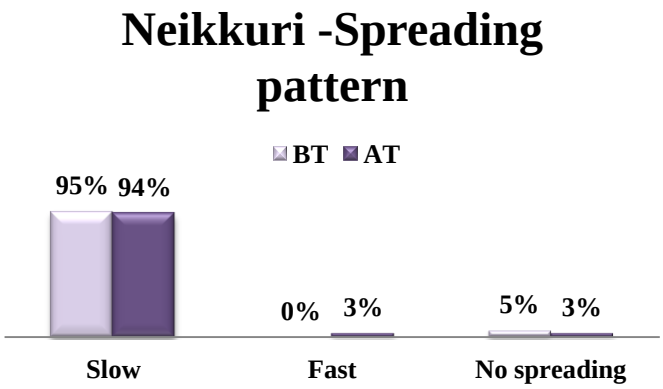
iv) Neikkuri (Oil-on-Urine Examination)

Fig 6.45 D: Observation of patients based on Neikuri shapes



In nei kuri urine samples on observation to shapes of the oil on urine, BT most of the 19 (51%) patients had ring shape, serpentine being 7 (19%) ,pearl being 2 (5%), mixed being 9 (24%) saladai being no patients (0%) and AT it was ring being 20 (54%), serpentine being 8 (21%), pearl being 1 (3%) , mixed being 7 (19%) and saladai being 1(3%). On observation to the spreading pattern of the oil on urine BT 35 (95 %) cases showed slow spreading pattern no patients (0%) showed fast spreading, 2 (3 %) patients showed no spreading and AT 35 (94%) patients showed slow spreading, 1 (3%) patient showed fast spreading and 1 (3%) patient showed no spreading pattern.

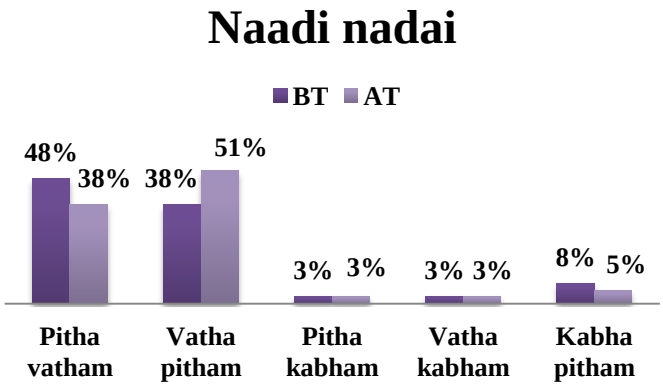
Fig 6.45 E: Observation of patients based on Neikuri spreading pattern



7) *Naadi nadai* (Pulse play)

Before treatment the naadi nadai of 18 (48%) of cases was pitha vatham, 14 (38%) of vatha pitham, 1 (3%) patient of pitha kabham and vatha kabham and 3 (8 %) patients were kabhapitham. After treatment 19 (51 %) of cases had vatha pitham, 14 (38%) of pitha vatham, 2(5%) patients of kabhapitham and 1 (3 %) patients of pitha kabham and vatha kabham naadi.

Fig 6.46: Observation of patients based on Naadi Nadai



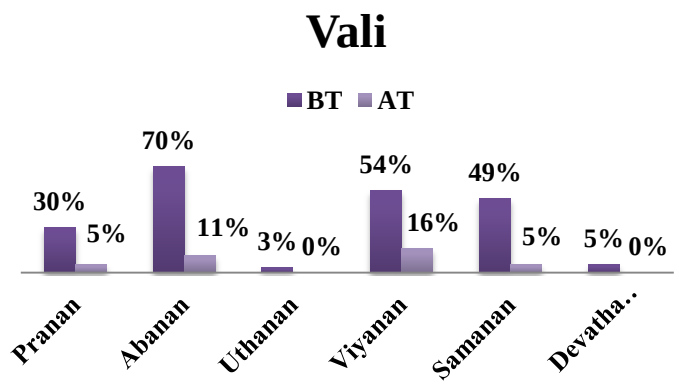
8) Uyir thathukkal (Functional constitution of the body) (n-37)

i) Deranged Vali (Bio energy movement)

Before treatment among 37 cases 30% of cases had deranged *pranan* (life air), 70% of *abanan* (downd ward air), 3 % of *uthanan* (upward air), 54% of *viyanan* (centrifugal air) , 49 % of *samanan* (digestive air) and 5% of *devathathan* (tiresome air) and after treatment it

was reduced to 5 % of *pranan*, 11 % of *abanan*, 16 % of *viyanan*, 5 % of *samanan* and 0% of *uthanan* and *devathathan*

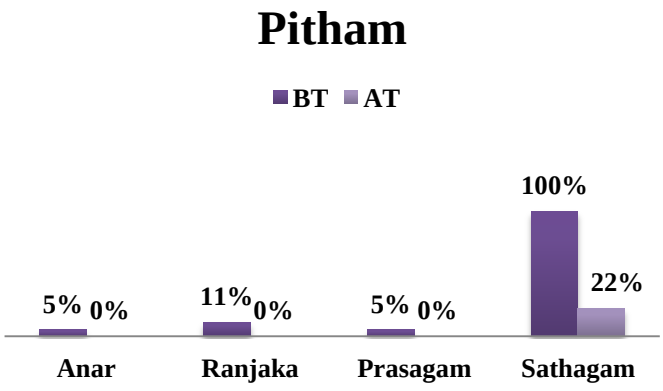
Fig 6.47 A: Observation of patients based on deranged Vali



ii)Deranged Azhal (Bio energy fire)

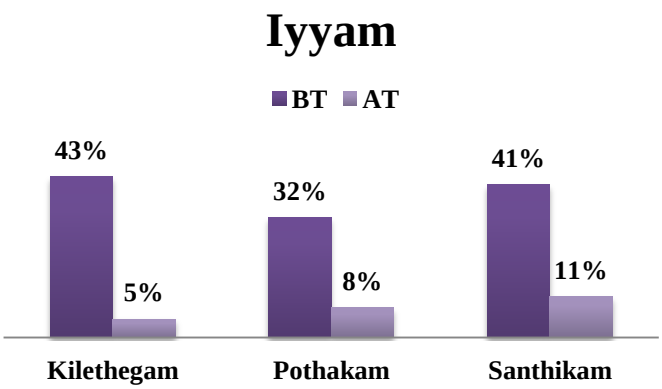
Before treatment 6 % of cases had deranged *anar pitham* (digestive fire) and *prasaga pitham* (complexion fire), 11 % of *ranjaka pitham* (haematinic fire) and 100% of *sathaga pitham* (accomplishment fire) and after treatment it was reduced to 0% of *anarpitham, ranjaka pitahm, prasaga pitham* and 22 % of *sathaga pitham.*

Fig 6.47 B: Observation of patients based on deranged Pitham



iii) Deranged Iyyam (Bio energy water)

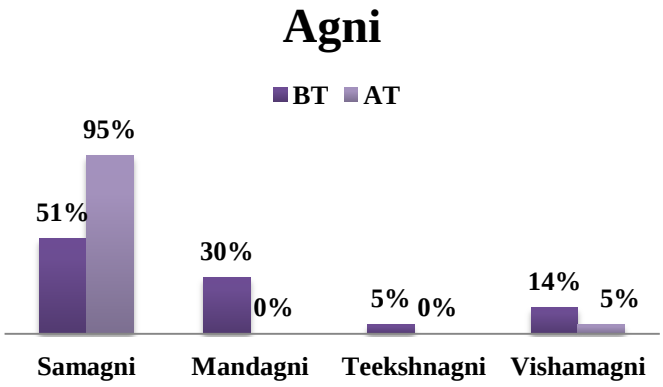
Fig 6.47 C: Observation of patients based on deranged Iyyam



Before treatment 43% of cases had deranged *kilethegam* (digestive iyyam), 32% had *pothakam* (gustatory iyyam) , 41% had *santhikam* (articular iyyam) and after treatment it was reduced to 5 % of *kilethegam*, 8% of *pothakam* and 11% of *santhikam.*

9) Pachakagni (Basal metabolic heat) (n-37)

Fig 6.48: Observation of patients based on deranged Agni



In the present study among 37 patients, BT majority of the patients, 19 (51%) belongs to samagni, 11 (30 %) patients belong to mandagni, 5 (14%) patients belongs to vishamagni and remaining 2 (5%) patients were found with teekshagni and AT 35 (95 %) patients belongs to samagni, 2 (5%) patients belongs to vishamagni.

V] Statistical Results

1) Safety parameters

i) Haematology

The mean value of Hb (15.33, 15.11) and T.WBC (7775.68, 7797.30) showed significant increase (P<0.001) both AT and AFU. ESR (6.22, 6.16) showed significant decrease (P<0.001) both AT and AFU. Platelets showed significant increase AT (2.89, 2.88, P<0.01**) and non significant changes (2.883, p > 0.05) AFU. RBC (5.28, 5.270, p > 0.05) showed non significant changes AT and AFU. All were within normal ranges.

Table 6.40A: Effect of CKC on haematological parameters in main clinical trial (AT)

Haematology	Mean		Mean diff	S.D	S.E	t	p
	BT	AT					
HB	14.797	15.33	0.5351	0.5017	0.0824	6.487	P<0.001***
ESR	9.62	6.22	3.405	3.158	0.519	6.560	
T.WBC	7451.35	7775.68	324.32	233.81	38.439	8.437	
RBC	5.233	5.281	0.0475	0.1911	0.0314	1.514	p < 0.05*
Platelets	2.840	2.894	0.0537	0.1287	0.02117	2.540	P<0.01**
Values are expressed as mean ±S.E.M, n=5, **P<0.01, **P<0.01, ***P<0.001							

Table 6.40 B: Effect of CKC on haematological parameters in main clinical trial (AFU)

Haematology	Mean		Mean diff	S.D	S.E	t	p
	BT	AFU					
HB	14.797	15.110	0.3135	0.33513	0.0550	5.690	P<0.001***
ESR	9.62	6.16	3.459	2.968	0.488	7.089	
T.WBC	7451.35	7797.30	345.94	353.23	58.071	5.957	
RBC	5.233	5.270	0.0367	0.15280	0.0251	1.463	p > 0.05
Platelets	2.840	2.883	0.0432	0.2034	0.0334	1.293	
Values are expressed as mean ±S.E.M, n=5, ***P<0.001							

Biochemical

The mean value of blood sugar (98.27,100.03), total cholesterol (174.57, 173.30) , LDL (77.89,76,81) VLDL (30.73,29.76), T.protein (6.10, 6.16), uric acid (4.03, 4.30), SGOT (24.73, 27.54) SGPT (25.43, 26.57) and SAP (147.46) showed significant decrease (P<0.001) both AT and AFU. HDL (41.65, 42.08) and TGL (123.70, 122.95) showed significant increase (P<0.001) both AT and AFU. Urea (22.22, 22.05), creatinine (0.819, 0.803) and total bilirubin (0.770, 0.768) showed non significant changes (p > 0.05) ATand AFU. All the parameters were within normal range.

Table 6.41A: Effect of CKC on biochemical parameters in main clinical trial (AT)

Biochemical Parameters	Mean		Mean diff	S.D	S.E	t	p
	BT	AT					
R.Sugar	102.03	98.27	3.757	5.361	0.881	4.262	P<0.001***
S.Cholesterol	179.65	174.57	5.081	1.164	0.191	26.558	
HDL	39.54	41.65	2.108	2.331	0.383	5.502	
LDL	88.57	77.89	10.676	1.733	0.285	37.473	
VLDL	41.27	30.73	10.541	2.641	0.434	24.273	
TGL	175.43	123.70	51.730	23.035	3.787	13.660	
B.Urea	21.65	22.22	0.568	2.620	0.431	1.318	p > 0.05*
S.Creatinine	0.819	0.819	0.000	0.0707	0.0116	0.000	
Uric acid	4.929	4.035	0.8945	0.3415	0.0561	15.933	P<0.001***
T.Bilirubin	0.7675	0.770	0.0027	0.0897	0.0147	0.183	p > 0.05
T.Protein	6.838	6.100	0.8380	0.4983	0.0819	11.810	P<0.001***
SGOT	32.78	24.73	8.054	1.794	0.295	27.305	
SGPT	33.84	25.43	5.243	2.712	0.446	11.759	
SAP	151.24	147.46	3.784	2.795	0.460	4.940	
Values are expressed as mean ±S.E.M, n=37 ; ***P<0.001							

Table 6.41 B: Effect of CKC on biochemical parameters in main clinical trial (AFU)

Biochemical Parameters	Mean		Mean diff	S.D	S.E	t	p
	BT	AFU					
R.Sugar	102.03	100.03	2.0	3.464	0.569	3.512	p<0.001***
S.Cholesterol	179.65	173.30	6.35	3.946	0.649	9.792	
HDL	39.54	42.08	2.54	3.015	0.496	5.126	
LDL	88.57	76.81	11.75	3.328	0.547	21.486	
VLDL	41.27	29.76	11.54	6.615	1.087	10.587	
TGL	175.43	122.95	52.48	32.510	5.345	9.820	
B.Urea	21.65	22.05	0.40	2.576	0.424	0.957	p > 0.05
S.Creatinine	0.819	0.803	0.00	0.070	0.011	0.000	
Uric acid	4.929	4.30	0.89	0.3415	0.056	15.933	p<0.001***
T.Bilirubin	0.7675	0.768	0.00	0.0897	0.015	0.000	p > 0.05
T.Protein	6.838	6.168	0.96	0.498	0.081	11.810	P<0.001***
SGOT	32.78	27.54	8.05	1.794	0.295	27.305	
SGPT	33.84	26.57	8.405	9.441	1.552	5.415	
SAP	151.24	148.97	3.784	3.334	0.548	6.902	
Values are expressed as mean ±S.E.M, n=37 ; ***P<0.001							

Semen parameters

The mean value of sperm count BT is 8.46 and significant (P<0.001) increase in the mean value of count (38.97, 28.97) was observed AT and AFU. The mean value of progressive motility BT is 10.19 and significant (P<0.001) increase was observed in the mean value of PR (36.03) AT and (34.59) AFU. The mean value of total motility BT is 24.05 and significant (P<0.001) increase was observed in the mean value of TM (54.54) AT and (48.43) AFU. The mean value of normal sperm BT is 23.70 and significant (P<0.001) increase was observed in the mean value of normal sperm (41.0) AT and (36.86) AFU. The mean value in the volume of semen BT is 1.31 and significant (P<0.001) increase in the mean value of volume (1.86) AT and (1.71) AFU was observed. The mean value in the liquification time of semen BT is 25.14 and non significant changes (p > 0.05) was observed in the liquification time both AT (23.65) and AFU (23.92).

Table 6.42 A: Effect of CKC on semen parameters in main clinical trial (AT)

Semen Analysis	Mean		Mean diff	S.D	S.E	t	p
	BT	AT					
Count	8.46	38.97	30.514	20.917	3.439	8.873	P<0.001***
Pr.Motility	10.19	36.03	25.838	11.656	1.916	13.484	
T.Motility	24.05	54.54	30.486	17.084	2.809	10.855	
Normal sperms	23.70	41.00	17.297	10.344	1.700	10.172	
Volume	1.311	1.865	0.5541	0.5108	0.0840	6.598	
Liquification	25.14	23.65	1.486	7.534	1.239	1.200	p > 0.05
Values are expressed as mean ±S.E.M, n=37 ; ***P<0.001							

Table 6.42 B: Effect of CKC on semen parameters in main clinical trial (AFU)

Semen Analysis	Mean		Mean diff	S.D	S.E	t	p
	BT	AFU					
Count	8.46	28.97	20.514	15.648	2.573	7.974	P<0.001***
Pr.Motility	10.19	34.59	24.405	11.725	1.928	12.661	
T.Motility	24.05	48.43	24.378	16.008	2.632	9.264	
Morphology	23.70	36.86	13.162	9.311	1.531	8.599	
Volume	1.311	1.716	0.4054	0.5249	0.0863	4.698	
Liquification	25.14	23.92	1.216	6.709	1.103	1.103	p > 0.05
Values are expressed as mean ±S.E.M, n=37 ; ***P<0.001							

Harmone parameters

The mean value of testosterone, FSH and LH, BT were 4.57, 7.86 and 7.14 respectively.The mean value of testosterone (6.09, 5.90), FSH (9.18, 8.87) and LH (8.0, 7.86) showed significant increase (P<0.001) AT and AFU.

Table 6.43A: Effect of CKC on harmone parameters in main clinical trial (AT)

Harmone	Mean		Mean diff	S.D	S.E	t	p
	BT	AT					
Testosterone	4.57	6.09	1.51	0.281	0.046	32.68	P<0.001***
FSH	7.86	9.18	1.31	0.532	0.087	15.04	
LH	7.14	8.00	0.86	0.599	0.098	8.768	
Values are expressed as mean ±S.E.M, n=37 ; ***P<0.001							

Table 6.43 B: Effect of CKC on harmone parameters in main clinical trial (AFU)

Harmone	Mean		Mean diff	S.D	S.E	t	p
	BT	AT					
Testosterone	4.57	5.90	1.32	0.385	0.063	20.92	P<0.001***
FSH	7.86	8.87	1.00	0.445	0.073	13.78	
LH	7.14	7.86	0.71	0.626	0.103	6.96	
Values are expressed as mean ±S.E.M, n=37 ; ***P<0.001							

Outcome measures

I] Primary Outcome measure

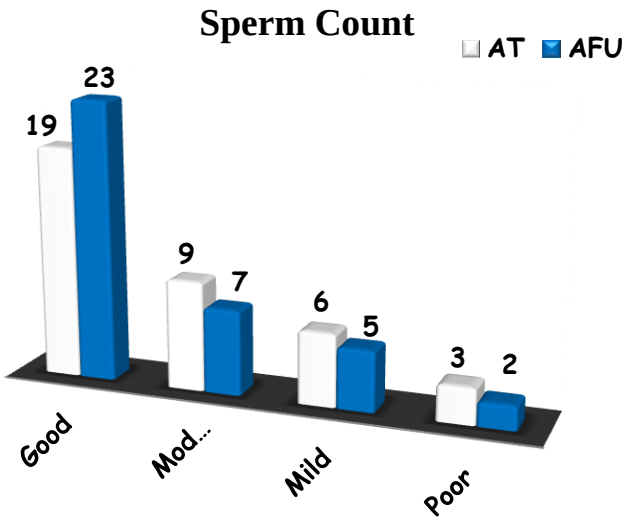
Table 6.44: Primary outcome measures

Response	Count n=37		Total Motility n=37		ProgressiveMotility n=37		Normal Sperms n=21	
	AT	AFU	AT	AFU	AT	AFU	AT	AFU
Good	19	23	23	18	20	16	12	8
Moderate	9	7	7	10	9	10	4	7
Mild	6	5	5	7	6	9	3	4
Poor	3	2	2	2	2	2	2	2

1) Sperm Count

On the basis of the criteria meant for the assessment of the sperm count it was observed that out of 37 patients, good response was observed in 19 patients AT and 15 patients AFU. 9 patients AT and 10 patients AFU showed moderate response, 6 patients AT and 8 patients AFU showed mild response and 3 patients AT and 2 patients AFU showed poor changes.

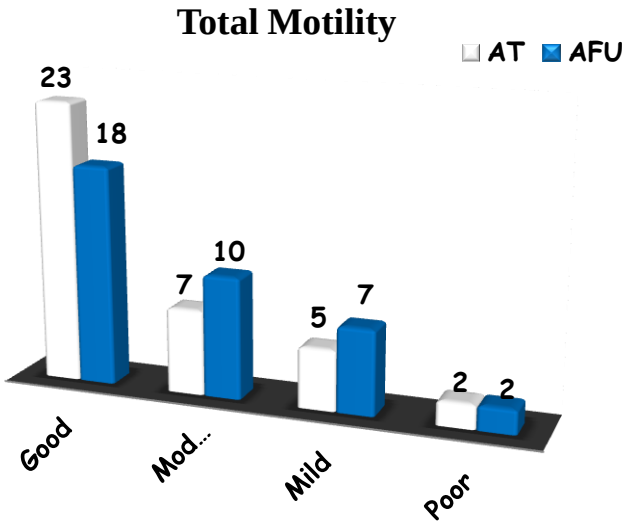
Figure 6.49A: Outcome measures of Sperm Count



2) Total Motility

For assessment of total motility, among 37 patients good response was observed in 23 patients AT and 18 patients AFU. 7 patients AT and 10 patients AFU showed moderate response, 5 patients AT and 7 patients AFU showed mild response and 2 patients both AT and AFU showed poor changes.

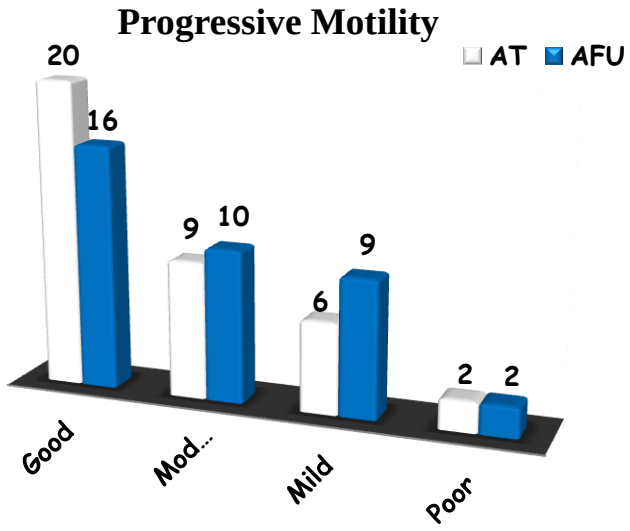
Figure 6.49B: Outcome measures of Total Motility



3) Progressive Motility

Among 37 patients, progressive motility showed good response in 20 patients AT and in 16 patients AFU. Moderate response was observed in 9 patients AT and 10 patients AFU.Mild response was observed in 6 patients AT and 9 patients AFU. 2 patients both AT and AFU showed poor changes.

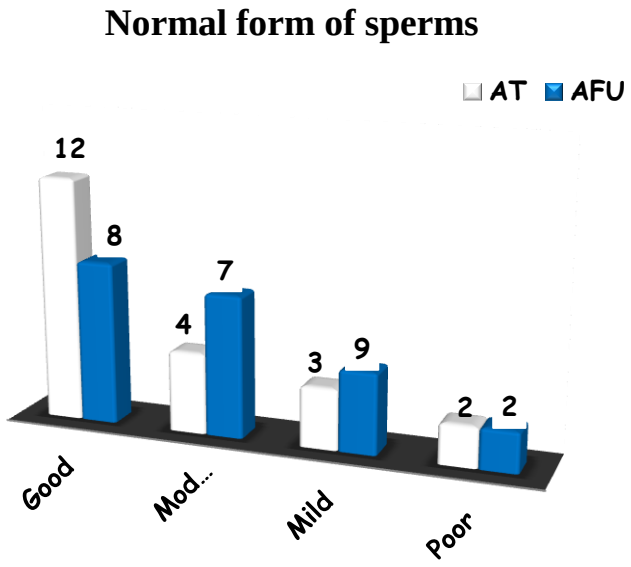
Figure 6.49 C: Outcome measures of Progressive Motility



4) Normal form of sperms

Among 21 patients, 12 patients showed normal form of sperm AT and 8 patients AFU. Moderate response was showed in 4 patients AT and 7 patients AFU. Mild response was obeserved in 3 patients AT and 4 patients AFU. In 2 patients poor response was showed both AT and AFU.

Figure 6.49 D: Outcome measures of Normal form of sperms



2] Secondary outcome measure

Testosterone, FSH and LH harmone showed significant increase ($P < 0.001$) after the treatment and at the end of the followup

7. DISCUSSION

Identification and Authentication of the Ingredients

Herbal drugs were identified and authenticated through visual inspection, organoleptic characters, morphology, micromorphology, taxonomical, microscopical and chemical methods elucidated by J.S.Gamble 2008 and Theodore cooke 2006. [221, 222]

Mineral drug (Gomutra silasathu) was identified and authenticated with reference to the microscopic characteristics.

Pharmacognostical study

According to World Health Organization the establishment of the macroscopic/microscopic characters of plant is the primary step in the direction to ascertain its identity and also the purity.[223] Literature review has exposed that seeds of *A.vasica* and *Alternanthera sessilis* have no description regarding the pharmacognostical studies and therefore the study was carried out on the samples (fresh seed).

Seed of the *Alternanthera sessilis* was 400µm-long; 250 µm- wide and 10µm-thick. Seed coat has well-known wings. Sclerotic- outer and the inner-seed coats; endosperm was large, polyhedral and thin walled.

A.vasica seed has inner- sarcotesta with outer-sclerotesta (40mm-thickness). Parenchymatous zone was thin at lateral part of seed which was developed into wider; many-layered at chalazal end. Radicle was circular in the sectional and the diameter is 550 µm. In powder microscopy study, oil bodies, starch grains, cotyledons, sarcotesta, and sclerotesta (ameboid within the outline) were observed. Physico chemical study in A.vasica seeds demonstrated loss on drying (at 105^0C) to be 6.80 percent, which might be due to the presence of fixed oil (in the seed) and moisture might be negligible. Total ash was observed to be 4.07 percent, which indicates the existence of inorganic content to be in lower amount. The extractive values (water soluble/ alcohol soluble) were evaluated to be 27.73 percent and 19.45 percent which indicates the existence of the high polar compounds.

In TLC study, three spots at UV 254 nm, four spots at 366 nm and four spots after derivatization were identified and in HPTLC study, eight peaks under 254 nm, two peaks under 366 nm and seven peaks at 540-nm were resolved. Spot in the R_f value - 0.89 was present in every conditions that might be due to the movement of the lower polar compounds which is near to solvent front. The anatomical characters demonstrated in *Adhatoda vasica*

and Alternanthera sessilis (seeds) may be used for the right botanical identification and authentication of the herbal materials and may be differentiated from its related species.

***Gomutra Silasathu parpam* [One of the 25 ingredients]**

Gomutra Silasathu parpam was indicated for nocturnal emission [*sopana skalitham*], veneral disease [*megam*], gonorrhoea [*premegam*], contagious ulcers [*koruku*] and glandular swelling [*kiranthi*]. In the preparation of the *parpam* (*silasathu parpam*) fermented rice water (*Kazhuneer kaadi*) was used for trituration [25]. *Kazhuneer kaadi*- rice water being kept for three hours and which taste sour since because of fermentation. [17] Rice has antioxidants like phenols-tocotrienols, tocopherols and γ-oryzanol. [224] Cow's urine was used for purification of *silasathu.* [82] Cow's urine is related to the nectar and it impedes the free radical formation. [225] It is rich in calcium, sodium, iron, potassium, phosphorus, copper, sulphur, chloride, nitrogen, lactose, phenols, gonadotropin, urinary proteins and vitamins like A;B;C; D and E.[226] It promotes and enhances the bioavailability of the drugs in combination treatment.[227] Cow's urine is able to cure diabetes, prostate, AIDS, cancer, and thyroid.[228] It has hypoglycemic, immunomodulatory and cardio respiratory effects. [229]

Siddha specifications on parpam were carried out to demonstrate the appropriate processing of the mineral asphaltum to the final *parpam*. In general the colour of the *parpam* must be white but occasionally will change according to the principal drug (Gomutra silasathu). Colour of the Gomutra silasathu parpam was found to be dark due to being as the principal ingredient.[205] It was odorless, tastless, lusterless, entered in to the crevices of the finger, not washed out from the crevices and floated on the surface of water. All these inferences showed that the *parpam* was well processed and have been prepared. *GSP* showed lower moisture percentage which is evident from the value of loss on drying being 2.50 percent. In *Siddha* text, the shelf life for *parpam* was refered to as 100 years [82] and *GSP* also might have the comparable shelf life. The total ash of *GSP* was evaluated as 77.45 percent which indicates that it includes 22.55 percent total combustible- organic matters. 23.75 % water soluble ash and 20.45 % acid insoluble ash reveals that 30.7 percent of total inorganic substance of *GSP* is water soluble and 26.4 percent of total-inorganic substance is acid insoluble in character. pH of *GSP* (10 % solution) was established to be 6.04 which denotes the slight acidic charater of the parpam .

ICP-OES study of *GSP* exposed that K (potassium) was identified to be high, calcium, Iron, sodium and magnesium were relatively in moderate quantity. Presence of

copper, zinc, manganese, chromium and nickel were revealed to be in less in quantity. Cadmium, Selenium, and tellurium were below the detection limit. For the achievement of reproduction selenium, zinc, nickel, copper, manganese and chromium are required. Ca, Mg and Cu are concerned in sperm motility. [50] Iron takes a vital part in spermatogenesis and in the normal role of testis. [230] Cadmium poison (acute) creates testicle injury and spermio-toxicity.[231]

The particle size study demonstrated that *GSP* accomplish its fineness. At higher temperature throughout the course in parpam processing, the possibility of the organic compounds are uncommon. [232] Regarding CHN analysis, the substance carbon in *GSP* was found to be 12.31 percent which showed that the organic particles derived from raw asphaltum remains in *GSP*. Nitrogen in *GSP* might indicate the presence of the amino acids in *GSP*. Organic constituents of asphaltum play in transporting the various mineral matters in to their cellular targets.[189] The presence of carbon in *GSP* might play an considerable place in increasing efficacy and making the drug biologically assimilable.[232]

Quality of the prepared Chandrakanthi choornam

All the purified drugs (herbal) and *GSP (Gomutra silasathu parpam)* was powdered then par boiled in milk (final purification process of *chooranam)* and dried under sunlight. Particle size study demonstrated that the drug *CKC* passes through mesh size of 100 which indicated its finess. The bacterial count and the fungal count were observed to be within the specified limits. The specific pathogens (*Salmonella* spp, *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) were known to be absent. The herbomineral formulation was established to be free from microbial contamination. Aflatoxins / mycotoxins (B1; B2; G1& G2) were observed to be BDL (below detection limit). Pesticide residues (organo chlorine / organo phosphorus) were found to be not detected in *CKC*. Heavy metals (mercury, lead, cadmium and arsenic) were found to be BDL (below detection level - 0.05 ppm). The results indicate that the drug *CKC* is of standard quality.

Preliminary phytochemicals of *CKC*

Phytochemical study showed the presence of steroids, amino acids, phenols, tannins, flavonoids, saponins, anthraquinones, glycosides and triterpenoids. Aminoacids are required in sperm activity.[82] Steroidal constituents (saponin, sterol) enhance the steroidogenesis and increase the androgen levels[233, 234] Phenol, [234] flavonoid, [235,21]and tannin [235] has antioxidant

activity. Presence of triterpenoids may be derived from asphaltum and showed to increase the testosterone and sperm count. [236] Anthraquinones is reported to have antioxidant activity .[237]

Physico-chemical parameters of *CKC*

8.45 percent of loss on drying (at 105°C) signifies that the drug *CKC* might have better shelf-life. 13.04 percent of total-ash may indicate that the *CKC* contains more inorganic substance, 3.39 percent of water soluble-ash may indicate that *CKC* contains significant quantity of the water soluble-inorganic substance. 5.61 percent of acid insoluble-ash in *CKC* might be due to *Gomutra silasathu*. The extraxtive values (19.25 percent of water soluble and 16.8 percent of alcohol soluble) demonstrate that *CKC* contains more polar compounds (tannins, glycosides, phenols, saponins and flavonoids) which is already proved from the phytochemical results. The pH value of 6.37 indicates that CKC is slightly acidic.

Heavy metal analysis of *CKC* (ICP-OES)

Metal contamination in pharmaceutical inventions creates serious hazard to human. Monitoring of metals in process intermediates and final products is an important activity in food and pharmaceutical industry. Metals act as the catalysts in disintegrating the pharmaceutical products. Sources for metal-impurities consist of i) those are added in the process ii) those are carried in the process iii) those comes from the process iv) occurs naturally (derived form plant or minerals). [238] Heavy metals (mercury, lead, cadmium and arsenic) were found to be below detection level in *CKC.*

Elemental analysis of *CKC* (ICP OES)

The quantities (ppm) of the elements are, calcium (6482.9), magnesium (1870), iron (988.6), zinc (21.98) and copper (8.09). Zinc improves sperm maturation and activation, preserves the germinative epithelium, regulates the enzyme activity and mediates the metabolic regulation (sperm), extremely concentrated in the tail (mature sperm) and is concerned in motility. Magnesium, calcium and copper are in ionic form in semen and abnormal levels might affect the spermatogenesis, maturation and motility of the sperm and fertilizing capacity.[239] Presence of Fe, Ca, Mg, Zn and Cu may possibly be responsible for the spermatogenic activity of *CKC*.

Chromatography techniques

In view of the fact that the herbal formulations are in complex nature, chromatographic techniques were generally employed to create fingerprint which is the profile of the mixture of constituents present in the herbal product. Finger-printing might be applied to recognize the plant, measure the active ingredients/ markers, and identify the impurities/ contaminants like herbicides. [240]

Thin Layer Chromatography of *CKC*

Thin Layer Chromatography has been mainly applied in the qualitative study of the herbal medicines, help to characterize and furthermore track the constituents visually (initial separation technique). This chromatographic technique offers the option of presenting the outcome as an image. Different colours of the corresponding individual bands perform an additional dimension. [240] Photo-documentation (TLC) of *CKC* at UV 254 nm revealed 5 visible green spots, with the major spots at R_f 0.24. At UV 366 nm showed 8 spots with major spots at R_f 0.41; 0.48 and 0.54. At 540 nm revealed seven spots with the major spots at R_f 0.17; 0.38; 0.44 and 0.58.

High Performance Thin Layer Chromatography

HPTLC study is uncomplicated to run, multiple-samples could be examined and compared on the similar plate. These features of HPTLC create it as a valuable analytical method for the quality control requirements and chiefly at the initial stage in testing. Previously was used for identification by American Herbal Pharmacopoeia. In adding with the visual estimation it may offer extreme rapid screening process of the complex samples. [240] The HPTLC - finger printing of the extract (choloroform) of *CKC* at UV 254nm revealed 7 peaks with major peak at R_f -0.24 and at UV-366nm revealed 9 peaks with major at R_f 0.48 and at 540 nm revealed 11 peaks with major peak at R_f -0.36.

Thermo gravimetric analysis

TGA curve provide information about the weight lost during heating a drug sample at a known temperature [241] and it is utilized to find out the total-weight change in a drug sample during the thermal treatments. 5.14% of the drug was decomposed at 120^0C, which might be due to the loss of moisture content present in the sample.

Toxicity study

Safety and the efficacy study depend on the methods adopted in the drug preparation and variation from the traditional knowledge (method) may not give the desired outcome.[242] Acute toxicity studies (initial assessment in toxic studies) present fast, significant information and may specify whether additional toxicity studies must be conducted. It gives information on the health risk that is possible to occur from the short-term experience to a drugs and is performed in all compounds. [243] Even drugs used in a longer period may produce chronic toxicological hazards but not have been known. WHO guidelines intends to specify the standard system in toxicological studies (non-clinical) associated in assessing the herbal medicine safety.

As per WHO- long term toxicity guidelines, groups must be given at least 3 dissimilar dose levels. One dose level (no effect dose) must not cause any toxic; one dose level which can produce over- toxic effects must be included and within this dose range additional dose should be added which may develop the possibility to observe the dose response-relationship for the toxic manifestations. Vehicle control group of the experimental animals should be included. As stated by WHO rules the period of test drug administration to animals depends on the estimated period of the clinical use. In case of repeated administration (clinical), between 1 to 6 months the administration time for toxicity study is from three to six months in rats [209] and hence 3 months [90days] duration was selected as long term toxicity treatment schedule.

Dose calculation [210]

Table 6.45: Dose calculation for rats in toxicity study

Dose for a rat weighing 200gm = Human absolute dose X conversion factor (Human to clinical)
Dose for a rat weighing 200gm = 12 X 0.018 = 0.216gm/200gm b.wt
Dose for a rat weighing 1kg = 1.08 gm/kg b.wt

- For acute toxicity study the higher dose selected was 10 TD (10.8 gm/kg b.wt). It was administerd two times to the rat (single dose)
- For longterm toxicity study three doses were selceted.

Table 6.46: Three dose levels in long term toxicity study

1 x Therapeutic dose = 1.08 gm/kg b.wt (1TD)
3 x Therapeutic dose = 3.24 gm/kg b.wt (3 TD)
5x Therapeutic dose = 5.4 gm/kg b.wt (5 TD)

In long term toxicity 5TD was selected as higher dose instead of 10 TD, to avoid stress that will be caused due to two times administration of the dose per day for 90 days.

Acute Toxicity Study

CKC was orally given at dose of 10 times the therapeutic dose (10 TD). Rats were observed for general conditions, signs of toxic symptoms and mortality for every hour during the first day with particular concentration given during the first 4 h and thereafter every day for 14 days. 10 TD dose level did not show any death, behavioural changes, toxic signs during 14 days and showed non significant changes in body weight when compared to that of the control group.On necropsy, no gross pathological abnormalities were observed in the vital organs and hence the acute toxicity study indicates that the drug is well tolerated up to 10 times [10.8gm/kg b.wt] the therapeutic dose in tested wistar rats.

Long term toxicity study

Three dose levels of 1.08gm/kg b.wt (TD); 3.24g/kg b.wt (3 TD) and 5.4 g / kg b.wt. (5TD) were administered to rats for duration of 90 days to determine whether *CKC* is toxic in long term use. The toxicity was assessed by estimating physiological, biochemical, hematological and histopathological effects of *CKC* on rats.

Feed and water intake

Diseases related with kidney, frequently are evident as water imbalance with the change in fluid intake subsequent to polyuria or oliguria. [244] Non significant changes were observed in the feed and water intake of animals between control and treated groups.

Weight

Body weight signifies the health status of the living beings. Increase in the body weight in rats specifies the normal health status of the animals and also about the information that no-degenerative alterations occur during the drug administration.[245] Vehicle control, 3 TD and 5TD group showed significant increase (weight) than the control group and TD group showed non significant changes in the body weight gain.

Organ weight

Absolute weights of brain, liver, stomach, kidney and thymus were found to be comparable with those of the control group rats. Increase in absolute weight of testis the main target organ (drug target) was noted in animals of 3 TD group which may indicate the androgenic effect. [246] Absolute weight of heart of 5TD group and lungs of vehicle and 5TD group was found to be significantly decreased ($p < 0.05$) however no cytoarchitectural changes of heart and lung tissues were observed in the histopathological studies of these group animals. All these finding implies the dose level of TD and 3TD does not have caused any toxic effect on all the vital organs

Blood examination

Blood examination is the excellent way in evaluating the health condition of animals since it plays an important role in the nutritional, physiological and pathological status of the organism.[247]

Hematology

Hematological index gives the blood status indication of the animals and are evaluated to assess the protein quality and the utilization. CBC (complete blood count) gives the information concerning the kinds/numbers of the cells in blood, chiefly RBC (red blood cells), WBC (white blood cells) and platelets. Complete blood count also assists in checking the fatique, bruising and weakness that might be present in the organism. Unusual high/ low counts might signify the presence of diseases like infection, anemia and other disorders. Changes in the qualitative/quantitative composition with the biochemistry of blood cells might be the warning and indication of a damaged function. [247] Haematological parameters might be used to find out the extent of harmful effect of the foreign compounds on blood. Thes investigations are reliable, accurate, highly sensitive and remains as the bedrock in ethical research, diagnosis, disease prevention and in treatment. [248]

MCV determines cell size (erythrocytes) and the capability of the rats to endure prolonged oxygen-starvation. Hgb (Haemoglobin) and haematocrit are the basic standards that reveals anemia (degree). MCHC is the index of average Hgb concentration of red cells. Low-RDW indicates the uniformity in the size of RBCs. Low- RBC indicates anemia which is caused by nutritional deficiency (iron deficiency, vit B12/ folate deficiency). Increase in the RBC indicates thallasaemia and increase in RBC with Hgb level is the inidicative of

polycythemia, generally caused by smoking, dehydration or genetic causes (changed oxygen sensing, deviation in Hgb oxygen release). [247]

White blood cells - Granulocytes (neutrophils; eosinophils; basophils), lymphocytes and the monocytes are concerned in protection of body against the foerign bodies and in the production of antibodies.[249] Increase in white blood cells indicates inflammation, hemorrhage and decrease in WBC indicates viral infection and bone marrow failure. Decrease in white blood cells indicates the decline in proportion of defense mechanism to fight againt infections. Increase in hemoglobin indicates lung diseases. Increased MCHC might be the end result in the prolonged-dehydration and decrease may indicate iron deficiency. Increase in Nu (neutrophil) indicates inflammation, hemorrhage and decrease indicates viral infection and bone marrow failure. Increase in lymphocytes might indicate viral infection, leukaemia and decrease might indicate bone marrow infiltration and lupus. Increased monocytes indicate leukemia, protozoan diseases or malignant diseases. Increased monocytes and WBC attributes to immune system of rats which attempts to detoxify the toxicity. Low platelets might cause the risk of excess bleeding/bruising. Increased platelet value might act as a marker in vascular diseases like micro-angiopathy. Increased lymphocytes indicate viral infection. [247]

Blood was evaluated for hematological toxicity of *CKC* and hemogram was estimated and results showed non significant changes in the haematological parameters like Hb, RBC, WBC, differential count ,MCH ,MCHC, Platelets, MPV, PCT , HCT,RDW and PDW of male rats of all doses levels (TD, 3TD, 5TD) of *CKC*. MCV value of vehicle control group - showed significant increase ($p < 0.05$) but within normal limits. The results do not indicate any serious pathological conditions.

Clinical Chemistry

The clinical blood chemistry values were used to analyze renal function (BUN and creatinine), liver function (total protein, albumin, globulin, AST and ALT), pancreas function (glucose), lipid assesment (Total cholesterol and triglycerides), serum electrolytes (Na, K, Cl)

Renal function test

Increase in blood urea and in non-protein level is observed in impaired renal function/acute renal fail-ure. [250] Serum creatinine indicates the condition of kidneys and it is the major catabolic result of muscles/ protein respectively. [247] Blood urea- was found to be significantly decreased ($P< 0.05$) in TD group which was comparable to the normal control

groups and serum creatinine found to be significantly increased (P< 0.05) in vehicle and TD group (within normal biological range). In 3TD and 5 TD it showed non sinificant changes. The renal parameters were within normal limits which rule out the renal function impairment.

Serum electrolytes

Serum electrolytes plays an vital role in controlling fluid levels, pH (acid-base balance), blood clotting, nerve conduction and muscle contraction. [246] Kidneys take part an vital role in the electrolyte regulation. [251] Electrolytes occur in extra and intra cellular fluids (serum sodium, chloride). Decrease in serum sodium and chloride below the accepted values indicates dehydration/shock. Low potassium values (below 3 mmol) are related to arrhythmia, tachycardia and cardiac arrest and high potassium indicates cardiac arrhythmia. [247]

The values of sodium of rats treated with all three dose levels were found to be comparable with those of the control group. Potassium showed significant decrease (P< 0.01) in 5TD group and non significant changes in the vehicle control, 1TD and 3TD groups. Chloride showed significant decrease (P< 0.01) in vehicle group, significant decrease (P< 0.05) in 3TD and 5TD group and nonsignificant changes in 1 TD group. All the serum electrolytes were within the clinical range.

Lipid Assessment

High cholesterol in blood is the main cause for cardiovascular disorders. High triglycerides and low lipoproteins are related to coronary artery disease. [252] Total cholesterol showed non significant changes in 5TD group, significant increase (P< 0.05) in vehicle and 3TD group, more significantly increased (P< 0.01) in TD group (within normal biological range). The values of trigycerides in rats treated with three dose levels (TD, 3TD and 5TD) were found to be comparable with those of the control group. The normal limits of cholesterol and trigycerides rule out the cardio vascular disorder and coronary artery diseases.

Blood Glucose

Diabetes mellitus (DM) is the disorder in which the body doesn't have the ability to metabolize CHO (carbohydrates) properly. The disease is described by the excess quantity of sugar in blood and urine. [253] The present study showed non significant changes in blood sugar level of vehicle and in all three dose level groups.

Liver function test

Liver is the important organs which is responsible in breaking all the 'poisons' which enters the body. Liver function test describes its function. The enzyme ALT (sensitive marker in liver cell damage) produced in the liver cells, increases in the condition when liver cells are inflamed/ death. When the cells are damaged, ALT escapes in to the blood and rises in the serum levels. AST is the state that can be related with the cellular necrosis of many tissues.[248]

AST- [Aspartate amino transferase] was increased significantly ($P< 0.05$) in 3TD and ($P< 0.01$) in vehicle group and showed non significat changes in 1 TD and 5TD groups. ALT [Alanine amino transferase] showed significant decrease ($P< 0.05$) in 3TD group and non significant changes in vehicle, 1TD and 5TD group. Albumin was increased significantly ($P< 0.05$) in 5TD and showed nonsignificant changes in rest of the dose levels. Globulin showed non significant changes in all the dose level groups. (Within normal limits)

Hyper-bilirubinaemia with no abnormalities in other LFT might result from increase in bilirubin formation, as in ineffective erythropoiesis or haemolysis and may be due to the inability in transporting bilirubin across liver (as in Gilbert's syndrome). [251] The values of bilirubin of rats treated with three dose levels (TD, 3TD and 5TD) and vehicle control were found to be comparable with those of the control group.

Total protein showed non significant changes in vehicle, 1 TD and 3TD groups and was increased significantly in ($P< 0.05$) 5TD group which doesn't fall under normal limits. The protein level in 5TD group can be substantiated by the results of the histopathological study of the liver and by further corroboration with abnormalities found in the organ weight of liver which revealed the non toxicological significance when compared to that of the control group. The cause for increase in total protein level is not known with assurance but might be due to the increase in hepatic protein synthesis/ decreased degradation. [244]

Taking these values in to consideration it may be inferred that the biochemical results of the therapeutic dose and three times the therapeutic dose was found to be safe.

Histopathology study

Histopathological examination of vital organs like brain, heart, liver, kidney, spleen, lungs, stomach, intestine, thymus and testis from both treated (1TD, 3TD, 5TD) and control (normal and vehicle) group animals showed normal architecture. Mild chronic gastritis was found in glandular part of the stomach in one animal of 5TD group (1/6). Psychological stress induces modifications of motility, secretion, visceral sensitivity, and local inflammatory responses in the GI tract. [254] The pathological change observed in one wistar rat in the higher dose group may be due to the psychological stress and it may be due to an individual variation.since such pathological changes was not found in other animals of that group, in which higher dose was given. Thus histopathological studies also confirmed the safety data, along with other physiological, biochemical and heamatological parameters after *CKC* treatment.

Together, these data suggest that the 90 days longterm toxicity study of *CKC* in rats at the 1TD and 3 TD dose level showed no-observed-adverse-effect-level (NOAEL) since all the animals survived till the end of the study and gross necropsy and histopathological results did not reveal any major findings. 5TD dose level showed adverse effect with reference to significant increase in total protein and significant decrease in the organ weight of heart and lungs.

SPERMATOGENIC ACTIVITY

Generally in male wistar rats spermatocytogenesis occurs at the age of 30 days while spermiogenesis begins at 50 days.[190] Spermatogenesis is the complex interplay which connects the structural components of testis and endocrine system.[234] Alcohol abuse is familiar in damaging the reproductive function in the experimental animals and as well as in human beings. Testis is extremely vulnerable to ethanol since it traverses the blood testis - barrier and lower the spermatogenesis. Alcohol abuse causes reduction in the production of testosterone, testis shrinkage, decrease sperm counts, abnormal shapes (sperm) and reduced sperm motility.Chronic ethanol abuse causes testicular atrophy and male infertility in alcoholic men. [98] To provide the scientific information on the androgenic and spermatogenic potentials of *Chandrakanthi chooranam* the study was carried out. Sperm parameters such as count, motility, viability, morphology, serum testosterone-levels, biochemical parameters like cholesterol, protein, glycogen, effect on body weight, ascessory sexual organ weight and histopathological studies on the tissues of testis, semniferius tubules and epididymis were

evaluated in *CKC* treated rats and compared with the control group rats and etnanol induced testicular injured rats.

Weight

Ethanol treated group showed significant decrease in the mean body weight changes of the rats when compared to that of the control group. Significant increase in the mean body weight changes was observed in study drug group when compared to ethanol induced group. Although ethanol supply above 50 percent of dietary energy (calories) it cannot be stored, and can not be used to preserve body weight.[98] Steroidogenesis (androgenic properties - androgens has anabolic effect) is one of the causes in the improved body and organ (sexual) weight in the treated groups. [138, 255]

Reproductive organ weight

Male reproductive development is synchronized by the mechanism of hypothalamus-pituitary-testis axis and the accessory sex organs. The increase in the weight of the accessory sexual organs (seminal vesicle/prostate gland) may be due the levels of the circulating androgen. [230] The weight of testis is related with the spermatogenic function and mainly depends on the number of undifferentiated sperm cells. [255] Generally in rats the number of leydig cells per testis increases parallely with the weight of the testis following the birth, which is accompanied with increase in the level of testosterone. [256] Androgens [testosterone] level is positively associated with the weight of testis, seminal vesicle, epididymis and prostate glands. [257]

The reduction in testicular weight of ethanol treated rats may be due to reduced tubule size, spermatogenic arrest and inhibition in steroid biosynthesis. Significant increase in the weight of the testis in the treated group (CKC) when compared to the induced group may be due to the restoration in the number of germ cells with respect to spermatogonia (which also includes spermatid and sperm) which is evident from the histopathalogical study [fig 6.34 A, B] Significant increase in the weight of the testis, seminal vesicle and prostate may be attributed to the increase in the testosterone level.

Serum testosterone

Testicular-Leydig cells are the chief site in the syntesis of testosterone. Steroid hormones play the main role in the continuation of spermatogenesis as well as fertility. Gonadotropins and TT (testosterone) are the principal regulators in germ cell maturity. Successful and entire male germ cell maturity depends on the endocrine interplay (balanced) of hypothalamus, pituitary and testis.[258] Ethanol considerably increase the lipid peroxidation in testis and reduces the conversion of dehydroepiandrosterone and also androstendione in to testosterone through decreasing the activity of 3b hydroxyl steroid dehydrogenase. [230]

High significant decrease in the testosterone was observed in ethanol groups when compared to the control group animals. Significant increase in the levels of testosterone in CKC treated group when compared with ethanol group may demonstrate the protection of germ cells and the sertoli cells [fig 6.34 A, B] and hence prevents the sperms and maintains its quality and quantity. [230]

Biochemical parameters

Protein

Ethanol inhibits the speed of hepatic protein metabolism [catabolism]. And which may be correlated to the amount of ethanol-induced oxidative stress.[259,260] In the spermatogenic study, in ethanol induced group the mean value of protein is increased which might be due to the oxidative stress (ethanol induced). The protein level in *CKC* group is comparable with the control group and lower than that of the indcued group which might be due to the presence of steroids and other antioxidants [flavonoids, phenol] in *CKC* which favours the protein metabolism by means of depressing the oxidative damage.[259]

Glycogen

Fructose is produced by seminal vesicle in the influence of TT (testosterone) and provides energy for the metabolism of sperm and its motility.[261] Fructose arises from glucose through glycogen-phosphohexose pathway (in the presence of phosphohexoisomerase / alkaline phosphatase).[262] Full-grown mammalian sperm have complete functional glycogen metabolism which results in the existence of glycogen/glycogen-like deposits in head/midpiece and will facilitate to maintain the energy and viability of sperm and increases the reproduction potential in mammals.[263] Testicular atrophy diminishes the androgen formation and causes decrease in level of fructose in the testis and epidydmis.[262] Significant

decrease ($p < 0.001$) in the mean values of glycogen was observed in the induced group (ethanol) than the control group may be due to the inhibition in the glycogen metabolism. Significant increase in the mean value of glycogen of the study group (*CKC)* when compared to the both ethanol and control group demonstrate the normal regulation in the glycogen metabolism which provides energy to sperm motility.

Cholesterol

Cholesterol is physiologically significant since it inhibits or stimulates the spermatogenesis and proceed as the precursor in the synthesis of androgen.[262] Study drug group (*CKC*) showed significant decrease ($p < 0.001$) in the mean value of cholesterol when compared to the ethanol induced group and control group which may be due to the utilization of cholesterol in the production of androgens and thereby in the stimulation of spermatogenesis.

Sperm parameters

Rats exposed to ethanol (25%) group showed significant decrease ($P<0.001$) in the sperm count, motility, normal forms of sperm and viabilty and significant increase ($P<0.001$) in abnormal forms of sperm; treatment group showed significant increase ($P<0.001$) in sperm count, motility, viability, normal form of sperm and significant decrease ($P<0.001$) in abnormal form of sperm when compared to that of the ethanol induced group.

In ethanol group, the sperm count was observed to be reduced significantly with reduction in the testicular weight and sperm motility which is an indication that ethanol had reduced/ inhibited spermatogenesis.[264] Low sperm count and reduction in the weight of testis signifies that the effect might be due to the leydig cell dysfunction and which results in the reduction in the secretion of testosterone which is responsible for diminished spermatogenesis and sperm counts.[265] Chronic consumption of ethanol reduces the motility and increases abnormal sperms (morphology) due to the injury / destruction in germ cells due to the free radical injury to the sperm cells.[230] Increase in the serum testosterone level, reproductive organ weights (testis, seminal vesicles, prostate gland), biochemical paramters (glycogen, protein, cholesterol) all demonstrate positive changes (increase) in the sperm count in the *CKC* treated group. Main function of epididymis is the maturation of sperm, that leads to the attainment of reproduction ability and sperm viability. [257] Animals treated with test drug showed normal morphology of epididymis [fig 6.36A, B] which may be responsible

for the increase in motility and viabilty of sperms. Administration of *CKC* attenuated the ethanol-induced decrease of sperm count, motility and viability.

Histopathology Study

Male infertility is greatly associated with certain histo-pathological characters of testes which include loss in sperm/spermatids, disarray/absence in germ cell layers[266] and hence histometric analysis of testis, epididymis and semniferous tubules and histogram of testis and semniferous tubules were evaluated.

Inside the testis, the most important target cells meant for toxicants which disrupt spermatogenesis are somatic-cells, (leydig/sertoli cells) and germ cells.[267] Histocytology of testicular tissue of the control group animals showed well differentiated germ cells with respect to spermatogonia. It was observed that the presence of mature somatic cells projects the perfect histomorphology of the testicular cells in this group. Normal sertoli cell was aligned properly on the basement membrane with oval dome shaped nucleus which demonstrates the normal morphology of the seminiferous tubule.

The Primary spermatocytes of the sample belonging to ethanol group showed condensed chromatin similarly the number of leptotenes and zygotenes are very minimal in this group. Leydig cell dysfunction occurs with testicular steroidogenic disorder[230] This is correlated with the decrease in the testosterone level and unclear leydig cells [fig 6.34 A, B] in the ethanol group rats. Decreased number of normal Sertoli cells with irregular cytoarchitecture and decrease in the number of spermatogenic cells into the lumen of seminiferous tubule was showed in the ethanol group [fig 6.34 B]. This observations may demonstrate the direct - toxic effect of ethanol on sertoli cells (which plays a vital role in spermatogenesis).

In *CKC* treated group primary spermatocytes with large centered nucleus and dense chromatin were observed. Leptotene and zygotene spermatocytes appeared rich in number. Preserverance of mature somatic cells were observed [fig 6.34 B]. *CKC* treated group showed many seminiferous tubules encircled by prominent membrane and also proper distribution of collagen fibers along with the seminiferous tubule. Tubules appeared to be uniform in size and shape. The seminiferous tubules showed normal spermatogenesis and spermiation [fig 6.34 A, B, 6.35 A,B, 6.36] This showed the steroidogenic activity of the test drug. Plenty of

sperms in seminiferous tubule evidently indicates the spermatogenesis regulated by hormone . [234] Leydig cells with clear-structure were observed. Presence of normal leydigs cells in this group is also suggestive of normal steroids synthesis which is evident from the comparable testosterone level with the control group. Sertoli cells provides nutritional support to spermatozoa.[267] Seminiferous tubules of *CKC* treated group showed plenty of sertoli cells with normal histology and also increased spermatogenic cells into the lumen of seminiferous tubule. Sertoli cells with apparent triangular nucleus was observed. Epididymis is the place where sperm maturation (morphology) takes place, which also provides the store house for matured sperms. [267] Animals treated with test drug showed numerous number of spermatid cells in the epididymis and also showed normal presence of epididymal lobule. Size and shape of the sperms appear normal in this group. Diameter of testis and semniferous tubules in the treated group was increased when compared with the control group and it showed prevention from the shrunken pattern when compared to the ethanol group.

Increase of sperm count and normal sperms (morphology) in the drug treated animals demonstrate positive changes in *spermatogenesis* and increase in sperm motility demonstrate positive changes of sperm maturation in epididymis. [257, 258]

Probable Mechanism

Antioxidant defense mechanism is of most important since peroxidative damage is considered as the significant cause for impaired-testicular function which results in wide range of pathological consequences (from testicular torsion- alcoholism). Normally the antioxidant system in the reproductive-tissues and the secretions are probable to quench ROS (reactive oxygen species) and defend against the oxidative injure to mature sperm and gonadal cells. [98]

In the present study rats treated with ethanol showed sertoli cells with irregular cytoarchitecture, decreased spermatogenic cells (in the lumen of seminiferous tubule), presence of high number of abnormal size and shape of the spermatozoa, reduction in the diameter of testis and semniferous tubules which may indicate the cytotoxicity. Reduction in count, motility increase in the abnormal sperms percentage is directly associated to infertility [153] Sperm membranes are mainly vulnerable to oxidative stress because of elevated poly-unsaturated fatty acids and have need of sertoli cell - barrier protection. [153] High cholesterol (high poly unsaturated fatty acids) level in the ethanol group might have damaged the sperm

membranes. And also the presence of sertoli cells with irregular cytoarchitecture in this ethanol group rats may indicate the impairment of sertoli cell barrier protection. These factors might results in the initiation of oxidative stress and reduces the spermconcentration, motility , viability and normal form of sperm in the ethanol group.

Sertoli cells plays an significant role in spermatogenesis.[267] The histopathology study results of *CKC* treated rats showed plenty of sertoli cells with normal histology, apparent triangular nucleus which might evidently indicate that the drug (*CKC*) has positive effect on spermatogenesis in rats which is also evident from the increased sperm concentration in *CKC* treated group. The androgenic activity of *CKC* is reflected by the increase in testis weight and serum testosterone levels.

Spermatogenic and androgenic effects of *CKC* is possibly due to the combined effects of the phytochemicals [amino acids, steroid, flavonoids, phenols, tannins, and saponins] and the nutritional elements [iron, calcium, magnesium, zinc, copper] present in *CKC*. Zinc enhances sperm maturation and motility, calcium, copper and magnesium on spermatogenesis[239] aminoacids in sperm activity, saponins and sterols in steroidogenesis; phenols, flavonoids and tannis shows antioxidative property. [234,235,21] The Antioxidants present in *CKC* might have ameliorated the oxidative stress induced by ethanol and had improved the androgenesis and spermatogenesis.

Clinical study

Environment, diet or lifestyle modification in current decades interfer with man's capability to produce sperm,[268] smoking and alcoholism directs to low semen quality.[9] Reproductive organs are extremely at risk to free radicals due to toxins (pesticides, insecticides lead, radiation, heavy metals) from environment.[268] Sperm count has been declining at two percent / annum for the last twenty years. This is due to increase of global temperature/ environmental pollution.[19] Oligozoospermia is the main cause for infertility in most of the couples. It is commonly treated by assisted reproductive techniques. Generally in these methods male with oligozoospermia are untreated, while the female partners are treated. Although various empirical treatments are in existence to cure oligozoospermia, it is hard to make out a therapy which is expected to help out a man with oligozoospermia. This lead to the trial of various therapies resultant in different success rates. [269]

Clinical study was carried out in to two phases

1] Pilot study and

2] Main clinical trial

The safety aspect of *CKC* was assessed on the basis of biochemical, haematological parameters and urinary parameters.In hematological analysis the parameters evaluated were Hb (gm/dl), Total RBC (cells/µl), Total WBC count (million/µl)), Erythrocyte sedimentation rate (ESR), Platelets (lakhs /µl). In biochemical analysis the parameters evaluated were blood glucose (mg/dl), serum total cholesterol (mg/dl), HDL (mg/dl), LDL(md/dl), VLDL (mg/dl), serum triglycerides (mg/dl), blood urea (mg/dl), serum creatinine (mg/dl), uric acid (mg/dl), serum total bilirubin (mg/dl),serum total protein (gm/dl), AST (IU/ml), ALT (IU/ml) and alkaline phosphatase (IU/L). In urine routine, the parameters evaluated were albumin, sugar, bile salts, bile pigments, Urobilinogen, Acetone and occult blood.

The efficacy aspect was assessed on the basis of semen parameters and harmone parameters. Semen examination was carried out at recommended standards of World Health Organization (WHO).The parameters evauated were semen volume [ml], liquification time [minutes], semen viscosity, sperm concentration [million/ml], percentage of progressive and total motile spermatozoa, percentage of normal forms of sperm and pus cells (per field). In Harmone analysis the serum samples were measured for testosterone, follicle stimulating hormone (FSH) by ECLIA method and luteinizing hormone (LH) by CLIA method.

Parameters were assessed at baseline (Day 0), at the end of the trial (Day 91) and at the end of the follow up (day 181).

Pilot study

This study was designed to assess the feasibility, safety and tolerability of administration of study in Oligozoospermic patients prior to conducting phase II main clinical trial. Study was conducted in 5 patients in the out patient department of National Institute of Siddha, Chennai, India. The trial drug provided high significant ($P<0.001$) increase in the sperm count, progressive motility, total motility and normal form of sperm both after treatment AT and AFU. The mean value of the safety parameters were within normal range both AT and AFU.

Main Clinical Trial

The study was conducted in accordance with the guidelines of the Indian Council for Medical Research (ICMR) and GCP adapted from ICH and accepted by Ayush. The study was conducted for one year in between April 2013 and April 2014 in the out patient department of National Institute of Siddha, Chennai, India.The Selected patients had been given *Agasthiar Kuzhambu* at the dose of 130 mg with ginger juice in the early morning in empty stomach to bring the mukutram to equilibrium. After that patients were put on 90 days of interventional Medicine 12 gm of *CKC chooranam* at night with milk. After the completion of treatment also the patients were kept under follow up for 3 months to asess the total overall effect of the treatment. Total number of patient's registered for study was 40. As per ICMR guidelines, Phase II is a therapeutic exploratory trials and normally 20 - 25 patients should be studied for assessment of each dosage. In this study 37 patients have completed the trial out of estimated 40 registered patients and their data were analyzed. No clinically significant adverse effect neither reported by the patients nor observed by the investigator through out the study period.

General status

According to previous illness the causes for spermatogenic arrest in 2 (5%) patients may be due to the history of mumps.[19] Socio economic status being the confounding factor also affect semen quality. 27 (73 %) patients comes under middle economic status and 7 (19%) patients of low economic status and 3 (8%) patients of high economic status.[4] Sleep disturbances is associated with the androgen deficiency [270] 16 (43%) had disturbed sleep and 12 (32%) patients were having delayed sleep. Increase in BMI is associated to low semen quality, diminished sperm couts and motility and increase in DNA fragmentation index. Obesity and overweight are normally associated with reduced testosterone levels. [271] BMI report of the main clinical trial signifies 2 (5 %) patients were underweight, 4 (11%) patients were over weight, 4 (11%) patients were obese and 27 (73%) comes under normal category.Majority of the patients 34 (92 %) were of mixed type and remaining 3 (8%) patients were vegetarian. Vegetarian diets may cause vitamin deficiencies (B12, A, B, C, E, Omega-3 fatty Acids, Se and Zn) and that might promote hinderance in the spermatogenesis process. Non- Vegetarian diet may cause Oligozoospermia since because of hormonal injections applied on poultries and cattles for the better yield. [88] Fish, meat, poultry, vegetables, various prepared and processed foods include considerable levels of MSG (free

glutamate). MSG (monosodium glutamate) has toxic effect on testes and causes oligozoospermia in rats. It causes testicular degeneration, hemorrhage and alteration. [272]

6 patients (16%) were addicted to smoking, 9 (24 %) patients to alcohol, 2 patients (6 %) to tobacco, 4 patients (11 %) both to smoking and alcohol. Smoking and alcohol are observed to cause testicular toxins/leyding toxins and causes oligozoospermia.[88] Gonadotoxins like chemotherapeutic agents; radiation exposure and various pharmaceutical agents (nitrofurantoin, cimetidine, ethanol, sulfasalazine, androgenic steroids and cannabis) acts as an direct spermato-toxins or via steroidal pathway. [48] In the clinical trial 10 (27 %) patients had history of exposure to gonadotoxic agents such as anti-bacterial drugs, drugs used for treatment of gastric problems, antidepressant drugs and steroids. [4, 55] It was observed that the maximum of 22 (59%) patients were found between the age group of 31 to 40 years , 11 (30 %) patients were between 21-30 years and 4 (11 %) patients were between 41-45 years. Highest proportion of duration of infertility was observed between 1-5 years in 23 (62%) patients, 8(22%) patients showed duration between 6-10 years, 5(13%) patients showed between 11-14yrs and in 1 (3%) patient the duration was found above 15 years. Other previous studies have mentioned highest percentage of male infertile age group between 30-39 years and duration between 1-5years. [273] 36 (97%) patients were presented with primary infertility and 1 (3%) patient complained of secondary infertility.In men rate of promiscuity increases when their partners are pregnant/breastfeeding and this describes the increased rate of secondary infertility through change in sperm quality with time. Causes may be fever, malignancy, testicular disease, stress, drug intake, environmental pollutants, surgical conditions and tobacco relating the reproductive tract.[48]

Rise in cortisol levels in reaction to stress reduce testosterone level[274] and may inhibit spermatogenesis.[4] In present study 5 (13%) patients were in depression and 10 (27%) were under stress. Excessive masturbation may lead to Oligospermia. [88] Antisperm antibodies might result as an exposure to sperm antigens in rectal mucosa and have been identified in the serum of homosexual men.[274] 21 (57%) patients comes under normal, 2 cases (5 %) were having perversion and history of masturbation before marriage in 14 cases (38 %) were reported. In this clinical study only 3 (8%) of the patients had the family history of delayed conception in mother or siblings, 1 (3%) patients had history of infertility in siblings and 33 (89%) patients had no family history. Majority of the patients 19 (51 %) were doing physical exertional work, 12 (32 %) patients were doing intellectual work, 02 (5 %) patients were

doing chemical /radiation work and 04 (11%) patients comes under thermal nature of work. Various occupational factors show deleterious result on male reproductive function. Welders, dyers, furnance and steel workers at their work place are exposed to the high temperatures and stated to have impaired spermatogenesis.[19] Ozone radiation and radio frequency electromagnetic waves emmited from the cell phones may lead to oxidative stress. Lead workers, professional drivers, agricultural workers and pesticide manufacturer are reported to have toxic effect on fertility. [4, 55, 57,58] Prevalence of infertility in male with blood group-O is higher than other blood groups (ABO), which shows the correlation between male inferility and blood group O. [275] This clinical study showed maximum of 15 (40%) patients reported in O+ group, 11 (30%) patients reported A+ group, 4 (11%) patients reported AB+ group and 7 (19%) patients reported B+ group. 15 (41%) patients were having the habit of hot water bath and 22 (59%) patients were having the habit of normal water bath. Sertoli cells and germ cells are highly susceptible to increased temperature which may cause partial/complete-spermatogenic arrest.[19] High summer temperatures, fever, saunas and frequent hot-baths results in destruction of germinal epithelium. Elevated testicular temperature (1°C beyond baseline) diminsh spermatogenesis (by 14%) and elevated testiculo-epididymal temperature diminish the sperm membrane coating protein synthesis. [19] 10 (27%) patients had primary education, 6 (16%) had secondary education, 18 (49%) had graduation and 3 (8%) were illliterate. Highest frequency of patients had done their graduations.

Safety study

Biochemical

Modification in liver function, exocrine pancreas and biliary system are common in DM (Diabetes Mellitus).[251] The mean value of blood sugar (98.27, 100.03) was significantly decreased (P<0.001) both AT and AFU. Significant decrease in bood sugar after treatment may be due to glucose which may be converted in to glycogen to provide energy for the sperm movement [263] which is evident from the significant increase (P<0.001) in the motility of the sperm and additionally the drug *CKC* is also given in polyuria. [25]

The mean value of total cholesterol (174.57, 173.30), LDL (77.89,76,81) VLDL (30.73,29.76) showed significant decrease (P<0.001) and HDL (41.65,42.08), TGL (123.70, 122.95) showed significant increase (P<0.001) both AT and AFU. The mean value showed no lipotoxicity.T.protein (6.10, 6.16), AST (24.73, 27.54), ALT (25.43, 26.57), SAP (147.46

) showed significant decrease (P<0.001), total bilirubin (0.770, 0.768) showed non significant changes (p > 0.05) both ATand AFU. The mean value showed no liver toxicity. Sterols reduces serum cholesterol by inhibiting the intestinal cholesterol-absorption; and polyphenols (flavonoids, tannins) lowers blood lipids.[250] The drug *CKC* has revealed the presence of sterol, flavonoids and tannins which may be the cause for the reduced cholesterol level after treatment and additionally the drug *CKC* is also given in biliousness [25](the composition of the bile is cholesterol; bileacids and phospholipids [251]). Urea (22.22, 22.05) and creatinine (0.819,0.803) showed non significant changes (p > 0.05) and uric acid (4.03,4.30) showed significant decrease (P<0.001) both AT and AFU. The mean value showed no renal toxicity.

Haematology

The mean value of Hb and T.WBC showed significant increase (P<0.001) in both AT and AFU. ESR showed significant decrease (P<0.001) both AT and AFU. Platelets showed significant increase AT (P<0.01) and non significant changes (p > 0.05) AFU. RBC (p > 0.05) showed non significant changes AT and AFU. All were within normal ranges. The evaluation showed absence of hematological toxicity .Copper along with iron is essential for the synthesis of haemoglobin. [276] *CKC* showed the presence of iron and copper (ICP-OES) which may be the cause for the increased Hb level after treatment.The increase in the WBC level may indicate the *CKC* in enhancing the immune system (immunopotentiating effect) after treatment. [250]

Efficacy study

Harmone parameters

Deficiency of testosterone and FSH (marker components) may cause oligozoospermia.[11] FSH is the main endocrine parameter to assess testicular function.[19] Testosterone will stimulate sertoli cells which will produce paracrine agent that stimulate sperm- proliferation/differentiation.[277] Plant testosterone are safer than the artificial form. [278]

BT 2 (5%) patients were having testosterone below 2.8 ng/ml, 34 (92%) were in normal range between 2.8 to 8 ng/ml, 1(3%) patients were having above 8 ng/ml. AT 36(97%) patients were improved to the normal range between 2.8 to 8ng/ml and 1 (3%) patient was having above 8ng/ml value.

BT FSH of 25(68%) patients were in the normal range between 1.7 to 8.6 mIu/ml and 12 (32%) patients were above 8.6 mIu/ml, AT same proportion was reported. BT LH of 34 (92%) patients were in the normal range between 1.5-12.4mIu/ml, 3(8%) patients were having LH above 12.4 mIu/ml. AT same proportion was reported.

The mean value of testosterone, FSH and LH, BT were 4.57, 7.86 and 7.14 respectively.The mean value of testosterone (6.09), FSH (9.18) and LH (8.0) showed significant increase (P<0.001) AT. Thus, it is proposed that the possible explanations for the increased sperm concentration and motility in the present study might be due to the significant increase (P<0.001) in the mean level of testosterone, FSH and LH .

Semen Parameters

Testosterone controls functional ability of accessory sex organs because sufficient seminal fluid is essential for sperm survival and motility. [19] Significant increase in the mean value of semen volume was observed after treatment which may be due to significant increase in testosterone level (AT) and in addition due to the presence of ingredients like *Tribulus terrestris* and *Madhuca longifolia* in *CKC* which has the property in increasing the semen seminal fluids. [72,109]

Non significant changes (p > 0.05) was observed in the liquification time after treatment. BT 6 (16 %) patients showed high viscous semen, 2 (5 %) patients of low visous semen and AT the values decreased to 1 (3%) patient in high viscous semen, no patients (0%) in low viscous semen.The *CKC* has the ingredient *Moringa oleifera* which is given in high and low viscous semen and *Bombax ceiba* which keeps the semen in suspension [40,17] which may be the cause for the improvement of normal viscous. BT 5 (13%) patients showed pus cells of more than value of 5, 4 (11%) patients showed less than 5 and AT the values decreased to 1 (3%) in more than 5, 4 (11%) patients in less than 5. The pus cell values decreased after treatment states the clinical improvement in observation of infection.

Sperm Parameters

Sperm count

Normal fertility needs normal spermatogenesis, epididymal storage and sperm transport. Partial/complete disruption to spermatogenesis effects in spermatogenic arrest and leads to oligozoospermia. Sertoli cells (functional/fully differentiated) are vital for spermatogenesis process and also offer structural/functional support to the growth/differentiate of germ cells.[19] The mean value of sperm count before treatment is 8.46 and significant (P<0.001) increase in the mean value of 38.97 was observed after treating with *CKC.*

Sperm motility

Male fertility depends on sperm motility and morphology.Yearly decline (2.6%; 0.3%; 0.7%) in sperm count, motility and morphology have been reported in earlier studies.[19] Sperm motility is the main factor used in evaluvating the sperm quality and this is obtained by the transist of sperm through epididymal duct. [271]

The mean value of PR (progressive motility) before treatmnet is 10.19 and significant (P<0.001) increase was observed in the mean value (36.03) of PR after treatment. The mean value of TM (total motility) before treatment is 24.05 and significant (P<0.001) increase was observed in the mean value (54) of TM after treatment. The observed result demonstrate the improvement in the quality of the sperm

Sperm morphology

Normal sperm morphology is required for progressive motility (linear) of sperm. Sperm morphology and motility are the excellent predictor for fertility potential.[19] The mean value of normal sperm before treatment is 23.70 and significant (P<0.001) increase was observed in the mean value (41) of sperm after treatment.

The significant increase in sperm count after treatment demonstrates *Chandrakanthi chooranam* have positive effect on spermatogenesis. Though the study mainly concern about sperm count, but still it was found that *CKC* has significant effect on motility and morphology of sperm. Histophysiology of epididymis and achievement of sperm motility depends in the presence of testosterone.[271] In the present study the percentage of progressive and total motility was significantly increased which is also comparable with significant increase in testosterone level. Viscosity interfer's with motility and sperm antibody coating.[44]

In this clinical study the observation in the improvement of viscosity, significant increase in motility and significant increase in WBC level (boosting the immune system) are interconnected with one another. Antisperm antibodies formation is an immune mediated system and steroids can suppress it.[279] *CKC* has steroids, ingredient like *Tribulus terrestris* (effective in anti-sperm antibodies) and has ingredients posessing immuno-modulatory activities like *Curculigo orchioides, Asphaltum punjabinum, Cinnamomum tamala,Vitis vinifera, Glycyrrhiza glabra, Phoenix dactilifera, Alternanthera sessilis* [98,189,118,148,179,157,194] which may be the cause for the improvement of motility and viscosity.This suggestion should be further explored through the interventional medicine in combating with the antisperm antibodies.

Clinical symptoms

Gonadal and the sexual dysfunction are linked with increased circulating-cortisol levels. Cortisol levels rises in stress response and cause drop in testosterone.[273] Nicotine causes negative impact on erectile function. Studies showed relationship between erectile dysfunction and BMI (increased in obesity) with hormone dysfunction.[48] Depression is connected with erectile dysfunction. Antibiotics, drugs used in treating blood pressure,gastric problems and CNS depressant interfere with production of sperm and ejaculation.[4]

Pitham is affected in nocturnal emmision. *Abanan [vatham]* is affected in premature ejaculation and *viyanan [vatham]* is affected in erectyle dysfunction. [32,41] Before treatment 20 (54%) patients with erectyle dysfunction, 26 (70%) patients with premature ejaculation and 11(30%) patients with nocturnal emission were reported and after treatment 6 (16%) patients with erectyle dysfunction, 4 (11%) patients with premature ejaculation and 2 (5%) patients with nocturnal emission were reported which bring about the study drug showed improvement in the clinical symptoms. The improvement in the symptoms after treatment may be due to the presence of *Tribulus terrestris, Moringa oleifera, Syzygium aromaticum* given in premature ejaculation; *Mucuna prurita, Tribulus terrestris, Curculigo orchioides, Glycyrrhiza glabra and cuminum cyminum* given in erectile dysfunction; *Mucuna prurita, Bombax ceiba ,Gomutra Silasathu parpam* given in nocturnal emission. [as stated earlier in drug review].

Follow up

After the completion of treatment also the patients were kept under follow up to observe further for 3 months, so that the total overall effect of treatment could be assessed. Investigations on safety study revealed that there was no alteration in any of the haematological and biochemical parameters related to any systemic toxicity AFU. The same significance P<0.001 persisted in the mean value of the count, total motility, progressive motility, normal form of sperms, semen volume and same non significant changes persisted in the the mean value of the liquification time of semen after follow up.

Probable Mechanism

Spermatogenesis pathway

Testosterone and FSH are hormones which act on Sertoli cells directly and promote spermatogenesis.[11] Hence, the probable reasons of the increased spermia, normal sperm count and motility in the present findings may be due the higher levels of testosterone which acts on the sertoli cells. [11] The drugs postive effect on spermatogenesis may be due to the presence of amino acids, iron, calcium, magnesium, zinc and copper. Aminoacids in sperm activity, Zinc in sperm maturation and motility, Ca, Cu, Mg and Fe in spermatogenesis. Additionally the ingredients like *Curculigo orchioides; Tribulus terrestris; Mucuna prurita; Asphaltum punjabinum; Moringa oleifera; Cinnamomum verum and Phoenix dactilifera* in *CKC* are reported to have spermatogenic activity and *Maerua arenaria; Cinnamomum tamala; Vitis vinifera; Bombax; Costus speciosus; Mesua ferrea; Glycyrrhiza glabra; Myristica fragrans; Ilicium verum; Cyperus rotundus* were therapeutically given in to increase sperm. [as stated earlier in drug review]

Steriodogenesis pathway

Luteinizing hormone and FSH (gonadotropins) acts on gonads which secretes testosterone. [280] LH stimulates leydig cells (in testis) to produce testosterone, while FSH acts on Sertoli cells to regulate the spermatogenesis. [280] Synthesis of ABP (androgen-binding protein) is FSH- dependent process. ABP binds testosterone with dihydrotestosterone and makes the availability of local androgenic-pool which supports gametogenesis.[278] In the present study there is significant increase in the concentration of testosterone, FSH and LH. The increase in LH concentration observed in this study may confer an increase in testosterone concentration. [280] The effect on the harmone parameters may be due to the presence of the steroid, glycoside and saponins in *CKC* which increase the steroidogenesis

and elevate androgen levels. Furthermore the androgenic effect of the drug may also be due to the androgenic activity reported in the ingredients like *Curculigo orchioides; Tribulus terrestris; Asphaltum punjabinum; Syzygium aromaticum; Costus speciosus and Ilicium verum*. [as stated earlier in drug review]

Endocrine pathway

Hormonal activity is completely an integrated process and hence it is essential to establish any probable endocrine impact. [281] Spermatogenesis is the complex-interplay connecting structural elements of testis with the endocrine system.[234] Psychological stress (chronic exposure) is known to produce various patho-physiological alterations in neuroendrocrine structure, resulting in changes in steroidogenesis. [210] The significant increase in serum LH and FSH levels may suggest stimulatory effect on pituitary gland which may demonstrate that the drug may act directly on hypothalamic– pituitary–gonadal axis. [280] This statement is supported by the action of the ingredients *Mucuna prurita; Asphaltum punjabinum; Curculigo present in CKC* on endocrine system. [as stated earlier in drug review]

Antioxidant pathway

Increased reactive oxygen species causes an imbalance between the ratio of oxidant and anti-oxidant, which in turn causes increased lipid peroxidation which damage sperm membrane and causes dysfunction. [210] Psychological stress is be associated with oxidant production [277] Smoking/passive inhalation of smoke cause seminal oxidative stress. Ethanol considerably enhances lipid peroxidation in testis and reduces the conversion of dehydroepiandrosterone/androstendione to testosterone.[259] Obesity increases sperm intra cellular reactive oxygen species [271] Antioxidants alters androgen level and increases the spermatogenesis.[234] *CKC* demonstrated to have phenols, flavonoids and tannis (antioxidants) and moreover the ingredients like *Curculigo orchioides; Tribulus terrestris; Mucuna prurita; Asphaltum punjabinum; Madhuca longifolia; Cuminum cyminum; Cinnamomum verum; Vitis vinifera; Bombax ceiba; Costus speciosus; Mesua ferrea; Glycyrrhiza glabra; Phoenix dactilifera; Myristica fragrans; Ilicium verum; Cyperus rotundus and Coscinium fenestratum* are reported to have antioxidant activities. [as stated earlier in drug review]

The mechanism by which the drug *Chandrakanthi chooranam* results in the improvement of spermatogenesis in human subjects should be further elucidated.

Outcome measures

On the basis of the criteria meant for the assessment of the sperm parameters it was observed that Good response was observed in 19 patients (count; n=37), 23 patients (total motility; n=37), 20 patients (progressive motility; n=27) and 12 patients (normal form of sperm; n=21). Moderate response was observed in 9 patients (count; n=37), 7 patients (total motility; n=37), 9 patients (progressive motility;n=27) and 4 patients (normal form of sperm; n=21). Mild response was observed in 6 patients (count; n=37), 5 patients (total motility; n=37), 6 patients (progressive motility; n=27) and 3 patients (normal form of sperm; n=21). Poor changes was observed in 3 patients (count;n=37), 2 patients (total motility and progressive motility; n=27 and normal form of sperm;n=21).

Sperm count, progressive motility, total motility and normal sperms showed significant increase (P<0.001) after treatment and the mean value differences (increase) after treatment were 30.51, 25.83, 30.48 and 17.29 respectively.Testosterone,FSH and LH showed significant increase (P<0.001) after treatment and the mean value differences (increase) after treatment were 1.51,1.31 and 0.86 respectively.

***Siddha* system of clincal study**

Kaalam (Age)

Among 37 cases, 54% of cases came under *vatha kaalam*, and 46 % of cases came under *pitha kaalam*. According to *siddha* text, *vathakalam* constitutes 1-33 years of age, *pitham kalam* 34-66 and *kaba kalam* from 67-99yrs.[32] In the present study male infertile patients with age between 21-45 years were included in the clinical trial. Hence all the cases selected for this study came under *Vatham* and *pitham kaalam*.

***Paruva Kaalam* (Season)**

Majority of 40% patients were reported during *Kaar kaalam* [rainy season - mid august to mid october] and followingly 30% of patients were reported during *Ilavenirkaalam* [early summer-mid april to mid june]

***Thinai* (Land)**

Majority of 15 (89 %) patients were reported from *neithal thinai* (costal tract) and 1 (3%) patient reported from *mullai thinai* [sylvan tract] in both of which *pitham* will be vitiated [32] which is opposite to the nature of sperm and 3 (8 %) patients reported from *kurinchi thinai* [hilly tract] which may causes disease regarding blood[32] which is the second

body constituent and the precursor in the formation of sperm. Study can be merely determined after a multicentric study.

Thega ilakkanam (Bio type)

Vali bio type groups are prone to *Thatu nashtam* (Oligozoospermia), iyyam bio type groups are prone to *Inthriya kuraivu* (Oligozoospermia), *Pitham* bio type groups are prone to *arpa sukilam* (Oligozoospermia)[32,36] This states that all the bio type are prone to oligozoospermia.In this clinical study majority of the patients were of *kabha pitham* biotype with the proportion of 30% , 24% of *vathapitham*,16% of *pitha kabham*, 14 % of *pitha vatham*, 8% of *vatham*, 5% of *vatha kabham* and 3 % of *kabham biotype*.

***Envagai Thervu* (Eight fold Examination)**

In *vali* derangement, tongue will exhibit sour taste, *in azhal kaipu* taste and in *iyyam* sweet taste will be exhibited. [32] Among 37 cases, before treatmnet 68% patients had normal taste 16 % patients had bitter taste, 8% patients with sour and sweet taste in their tongue which states *pitham* is affected maximum and after treatment 92% patients had normal taste, 5% patients with bitter taste, 0% with sour taste and 3 % patient with sweet taste in the tongue.

Before treatment 5% of patients were with affected skin colour (pallor) indicates *pitham* affected and after treatment all the patients were with normal skin colour.*Pitham* lies in blood and chyle. Excess chyme resulting in paleness of the body and decreased blood resulting in discoloration of the skin.[32]

Before treatment 14% patients had affected eye (pallor, burning sensation) which indicates the vitiation of *iyyam* and *pitham* and after treatment it was normal.Before treatment in 19% of patients faeces were affected (constipation) which indicates the vitiation of *vatham* and after treatment 3% of patient faeces were affected and 97% patient's faeces were normal. As per *sathaga naadi*, pulse perceived in thatunashtam [oligozoospermia] are *vali naadi, vali azhal naadi, azhal vali naadi and pithathil vayu*.[32] Before treatment the *naadi nadai* of 48% cases was *pitha vatham*, 38% of *vatha pitham*, 3% patient of *pitha kabham* and *vatha kabham* and 8 % patients were *kabhapitham*. After treatment 51 % of cases had *vatha pitham*, 14 38% of *pitha vatham*, 5% of patients were *kabhapitham* and 3 % of patients with *pitha kabham* and *vatha kabham naadi* were reported.

BT 34 (92%) patients with normal voice, 3 (8%) patients with affected voice (*Thantha oli*) and AT the same proportion was reported. Both before and after treatment all the patients were with normal *meikuri* (normal body temperature, no tenderness). In the present clinical study datas of *mozhi* and *meikuri* does not have research significance as majority of them are normal.

Before treatment 76 % of patients had normal urine colour, 89% of normal smell, 43% of frothy urine, 46% of deposits, 86 % of normal specific gravity and volume and after treatment 89 % of normal urine colour, 97 % of normal smell, 19% frothy urine, 11% of deposists, 97% of specific gravity and volume were reported. Frothy micturation is one of the features of *aan maladu*. As per siddha science frothiness is due to *kabam* derangement. [32]

In *nei kuri* urine samples on observation with regard to shapes of the oil on urine, before treatment 51% patients had ring shape, serpentine being 19% ,pearl being 5%, mixed being 24%, 0% of case with *saladai* and after treatment ring being 54%, serpentine being 21%, pearl being 3%, mixed being 19% and *saladai* being 3%. Pearl and *saladai* are incurable signs. Ring, serpentine and mixed are curable signs. [32] On observation to the spreading pattern, before treatment 95% cases showed slow spreading pattern, 0% of patient showed fast spreading pattern, 3 % of patients showed no spreading and after treatment 94% of patients showed slow spreading, 3% of patient showed fast spreading and 3% of patient showed no spreading pattern. Slow spreading is curable; fast and no spreading are incurable signs. [32] The results shows the clinical improvement.

***Uyir thathukkal* (Functional constitution of the body)**

Deranged *Vali* (Bio energy movement)

Pranan [life force] is responsible for respiration and digestion. *Abanan* [downward air] responsible for absorption, assimilation of essence, excretion of urine, faeces, ejection of semen, contracts and relaxes the sphincters. *Uthanan* [upward air] responsible for speech, stations the essence of food at appropriate place (nutrition). Helps in the digestion of food. *Viyanan* [centrifugal air] disseminates all over the body through vessels and nerves causing voluntary and involuntary functions, takes the essence of food to all the parts of the body. *Samanan* [digestive/homeostatic air] balances other components, responsible for assimilation, equalizes six tastes, water, food etc. *Devathathan* [Tiresome air] responsible for lassitude, laziness and lethargy. [32] Before treatment among 37 cases 30% of cases had deranged

pranan, 70% of *abanan,* 3 % of *uthanan,* 54% of *viyanan*, 49 % of *samanan* and 5% of *devathathan* and after treatment it was reduced to 5 % of *pranan*, 11 % of *abanan*, 16 % of *viyanan*, 5 % of *samanan* and 0% of *uthanan* and *devathathan*

Deranged *Azhal* (Bio energy fire)

Before treatment 5 % of cases had deranged *anar pitham* and *prasaga pitham*, 11 % of *ranjaka pitham* and 100% of *sathaga pitham* and after treatment it was reduced to 0% of *anarpitham, ranjaka pitahm, prasaga pitham* and 22 % of *sathaga pitham*. *Ranjaka pitham* (Haematinic fire) exists in the stomach, stains red the essence of the digested food. *Anar pitham* (digestive fire) exists in stomach and intestines possess the quality of increased fire dries up water contents of the food stuffs and digests all the ingested foods. *Prasaga pitham* (complexion fire) existing in the skin, it gives lustre to it. *Sathaga pitham* (accomplishment fire) existing in the heart, with the help of knowledge, intellect and affinity performs the desired act.[32,27]

Deranged *Iyyam* (Bio energy water)

Before treatment 43% of cases had deranged *kilethegam,* 32% had *pothakam,* 41% had *santhikam* and after treatment it was reduced to 5 % of *kilethegam,* 8% of *pothakam* and 11% of *santhikam. Kilethegam* (digestive iyyam) exist in the stomach, break down the ingested hard food stuffs and liquifies them. *Pothakam* (gustatory iyyam) exists in the tongue, intimates the taste of the food. [32,27]

***Udal Thathukkal* (Physical constituents)**

Excess chyme results in diminished digestive fire, fatigue, excessive sleep, slackening of all the joints, heaviness of the body, paleness and coldness,excess salivation and decreased chyme results in skin roughness, body pain,leanness. Blood excess results in dyspepsia, reddening of eyes and skinand decreased blood causes liking of cold and sour foods, nervine debility, dryness and skin discoloration. Excess muscle causes increases of flesh in cheeks, stomach, thigh, penis and neck and decreased muscle causes five sense organs weakness, joint pain, shrinking of chin, buttocks, penis and thighs. Excess fat causes symptoms similar to that of muscle excess, along with fatigue, dyspnoea on exertion, associated with excess muscle formation in buttocks, genitals, chest, abdomen and thighs and decreased fat causes the weakness of hip with pain, emaciation of the body, enlarged spleen. Excess bone causes increased bone growth, teeth and decreased bone causes painful joints, loosening of teeth,

fissures and falling of nails, splitting and falling of hairs. Excess marrow causes obesity, heaviness in the eyes, swelling in finger and toe joints, decreased urination, gradually healing ulcers and decreased marrow casues perforation in bones, shock and diminished vision. Increased semen causes increased libido and fromation of renal calculi and decreased semen causes dropy ejaculation of semen or blood during copulation, pricking pain in the testis, inflammation and blackening of the genitalia. [32,27]

Before treatment 100% patients had deranged *saaram* (chyle) and *sukkilam* (semen), 76% patients had deranged *senneer* (blood), 32% patients had deranged *oon* (muscle), 27% patients had deranged *kozhuppu* (fat), 41% patients had deranged *enbu* (bone), 30% patients had deranged *moolai* (bone marrow) and after treatment it was reduced to 22% of *saaram* and *sukkilam*, 8% of *senneer*, 5% of *oon* and *kozhuppu*, 11% of *enbu*, 3% of *moolai* .

Status of *Pachakagni*

Samagni (optimal digestive fire) is constituted by *samanan, analam and kilethakam*. It is the digestive fire which ensures proper and timely digestion of all the solid and liquid food materials taken by an individual. *Vishamagni* (Toxic digestive fire) is delayed digestion due to deranged and dispalced *samanan* leading to toxic digestion. *Teekshagni* (Fiery digestion) is due to increased digestive fire intake of even improperly cooked/under cooked food gets digested along with essence. *Mandagni* (Sluggish/delayed digestion) in which without digesting immediately the food items taken eagerly, it produces rumbling noise in the abdomen along with abdominal distension and heaviness of body. [32, 27]

In the present study among 37 patients, before treatment majority of the patients (51%) belongs to *samagni*, 30% patients belongs to *mandagni*, 14% patients belongs to *vishamagni* and 5% patients were found with *teekshagni* and after treatment 95 % patients belongs to *samagni*, 5% patients belongs to *vishamagni*. Irregular pattern in taking food, starvation, poor eating, over eating, incompatible food and taking unwholesome diet finally will results in mandagni, teekshagni, vishamagni which will actually results in incomplete nourishment of *saaram* (precursor in the formation of sperm).

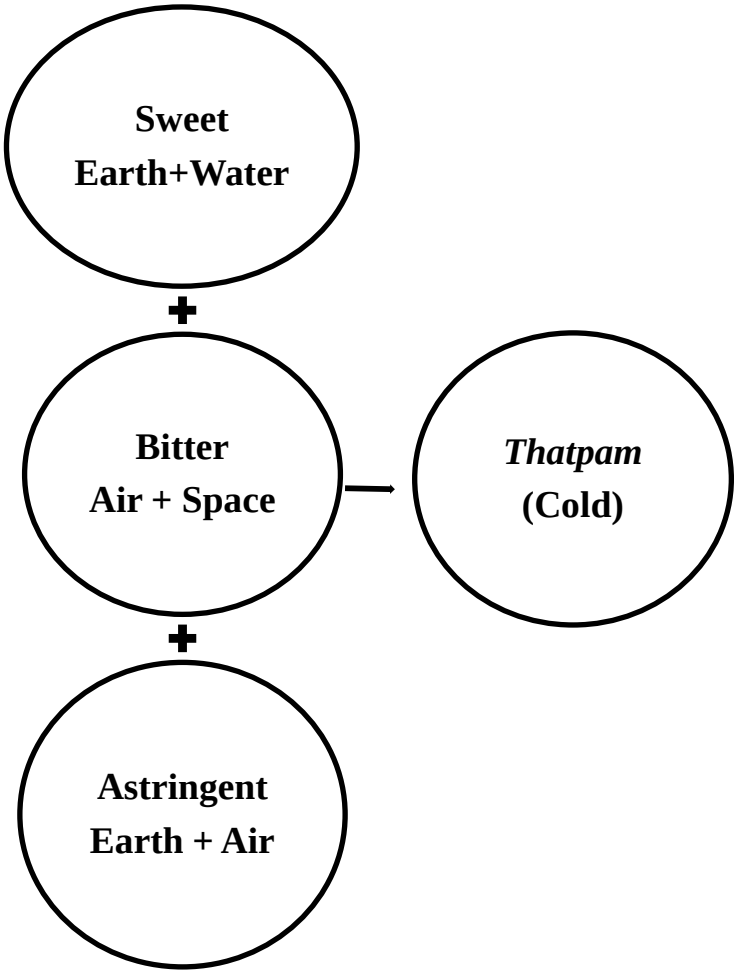
Siddha* – Probable mode of actions of *Chandrakanthi chooranam

***Suvai* (Taste)** [30,32,36,39]

Sweet	Strengthens semen/sperm [*Inthirya balam*]
Bitter	Toxin removal [*nanju neekum* - ROS, free radicals]
Astringent	Purifies the blood, and reduce cholesterol

***Viriyam* (Potency)**

Viriyam of CKC is cold [*thatpam*] and will reduce heat (*pitham*)



***Vipakam* (Post digestive transformation of tastes)**

A concept explaining the disintegration/assimilation of six taste in the digestive tract in to three primary taste. Sweet and salt becomes sweet; sour becomes sour; bitter, pungent and astringent becomes pungent. *Chooranam* will be digested in 12 *naligai* [288mts]. Vipakam of CKC is sweet and pungent.

***Prabhavam* (Specific action)**

Opposite action to the original character of the drug's taste, action, potency, post digestive transformation.

Astringent / bitter / sweet taste of *CKC* will balance *Pitham*

General action of the drug

Asphaltum punjabinum will act as a synergistic enhancer of other drugs and enhances the bioavailability in the body, tonifies the action of the seven body constiuents (plasma, blood, muscle, fat, bone, marrow and semen). Removes body toxins, transports mineral substances to their target cell and nutrients into the deeper tissues. [185] *Madhuca longifolia* will promote active components extraction from herbs and absorbs active components from gastrointestinal tract.[112]

By correcting *vatham, pitham* and *kabam*, nourishing the seven body constituents, the drug may improve the sperm parameters [count, motility and normal form of sperms].

***Siddha* treatment Method [45]**

Oppurai	A cure employed by the drugs which stimulate the symptoms similar to those of the disease. Heat to heat and cold to cold.
Ethirurai	A cure by those drugs which acts against and supress the symptoms of the disease.
Kalapurai	Combined action of *oppurai and ethirurai*

`Sweet taste and *seetham veeriyam* will reduce *pitham*, bitter will subside *pitham* which comes under *ethirurai*. Sweet will increase sperm production which comes under *oppurai*.And hence the treatment method of *Chandrakanthi chooranam* comes under *kalapurai*.

***Siddha* interpretation in three-humour**

- 51% patient were doing physical exertional work which causes vitiation of *Vatham* since *Vayu* [air element] governs the activities like running, sitting,walking, lying and standing.[32,27] 16% patients were getting a continuous exposure to *Pitham* vitiating causes like working under thermal exposure, chemical, radiation work. *Theyu* [fire element] has the phenomena with qualities of heat, burning [32,27] *Pitham* is directly opposite to the quality of sperm.

- *Iyyam* lies in sperm, adipose tissue, blood, bone and bone marrow. *Vali* lies in muscle. *Pitham* lies in blood and chyle.[32] In observation of *udal thatu* in all the patients *Pitham* and *iyyam* is vitiated and 32% of patients *vatham* is vitiated.

- 43% of patients had unhealthy sexual habits, 75% of the people had sleep disturbances, 15 (41%) patients were having the habit of hot water bath which comes under the vitiation of *pitham* since because of the qualities of fire element.[32]

- 57% of patients were addicted to smoking, alcohol and tobacco. Alcohol is *thamo gunam (Vatha)* food articles[30] and hence causes derangment of *vali*. Nicotiana tabacum (Tobacco, smoking) vitiate *pitham* [40] and destroys sperm.

- 5 (13%) patients were in depression, 8 (22%) patients were in anxious state, 4 (11%) patients were in fear and anger state each and 10 (27%) were under stress. These factors vitiate *vatham* which has the quality of mental agony and *pitham* which has the quality of fear. [32,27]

- Among 37 cases BT 6 (16 %) patients had bitter taste in their tongue which states *pitham* is affected, 3 patients (8%) with sour taste which states *vatham* is affected and sweet taste in their tongue which states *iyyam* is affected. [32]

- BT 2 (5%) patients with affected skin colour (pallor) and 5 (14%) patients with affected eye (pallor, burning sensation) which states *pitham* is affected and 7 (19%) patients faeces were affected (constipation) which states *vatham* is vitiated. [32]

- BT most of the 19 (51%) patients had ring shape with *pitham* being vitiated, 7 (19%) patients with serpentine with *vatham* being vitiated, pearl being 2 (5%) with *iyyam* being vitiated, mixed being 9 (24%) *thontham* kutram is reported. [32]

- Before treatment the *naadi nadai* of 18 (48%) of cases was *pitha vatham*, 1 (3%) patient of *pitha kabham* in which *pitham* is being dominant.14 (38%) patients of *vatha pitham*, 1 (3%) patient of *vatha kabham* in which *vatham* being dominant and 3 (8 %) patients were *kabhapitham* in which *kabam* being dominantly vitiated. [32]

- On the basis of observation, it may be concluded that *pitham* and *vatham* are the prime predisposing factor and *iyyam* being minor factor for causing Oligozoospermia and after treatment improvement in the sperm parameters were observed due to alleviation of the vitiated humours by the trial drug.

***Siddha* interpretation in body constituent**

As stated by *siddha* physiology seven body constituents has realtion with one another Ingested food will be transformed to chyle which is proposed to develop into blood. The RBC'S in blood will carry oxygen and will supply to muscle cells which make use of the oxygen in breaking the glycogen in muscle and will produce ATP to provide energy. Pyurvic acid will be the end product of this glycolysis and acetyl coA formed from pyurvic acid will supply carbon atoms for synthesis of cholesterol. Cholesterol is the precursor in producing steroid harmones. Steroid harmones will procced on the osteoblast/osteoclast and modifies bone resorption/formation. Inside the inner region of bones, bone-marrow is situated which consist of fat cells, fluid, fibrous tissue, blood vessels and hematopoeietic cells. And from this bone marrow semen is formed. Recent research had showed early-stage sperm cells created from human bone marrow. [77]

***Saaram* [First precursor in the formation of sperm]**

Saaram gives mental and physical perseverance. *Samagni* is the optimal digestive fire constituted by *samanan, anila pitham and kilethakam*. It is the digestive fire which ensures proper and timely digestion and it is the heat required for nourishment of life. Any imbalance in samagni will cause toxic, fiery or delayed digestion and will cause improper nourishment of *saaram*. *Pitham* which has the quality of fear will be vitiated in psychological stress and affects saaram which gives mental perseverance. Emotional stress will reduce testosterone

levels and will interrupt spermatogenesis. [274] The autonomic nervous system and adrenal hormones plays role in stress response, which will also affect steroidogenesis / spermatogenesis .The sweet taste in *CKC* will pacify the *pitham thodam* and will balance the *samagni* and thus will noursih *saaram*. The ingredients like *Cuminum cyminum; Vitis vinifera; Costus speciosus; Glycyrrhiza glabra; Lawsonia inermis; Mucuna pruriens; Shilajith* which is reported to have antistress and antidepressant activity [as stated earlier in drug review] reduces stress and will nourish *saaram.*

Blood [Second precursor in the formation of sperm]

Significant increase in the Hb level after treatment is reported and which may be due to the presence of iron and copper in CKC. [276] Moreover the ingredients like *Madhuca longifolia; Cuminum cyminum; Vitis vinifera; Myristica fragrans* [as refered in drug review] will enrich the blood and *thuvarpu* taste in *CKC* helps in the formation of-blood[39] Altogether may demonstrate that the second physical constituent blood is well nourished.

Muscle [Third precursor in the formation of sperm]

Glycogen is stored in muscles & liver. Sweet taste has the action of strengthening semen/sperm and according to modern science metabolism of carbohydrates [sweet taste] particularly glucose, is essential for male reproductive health. Maintenance of testicular-glucose metabolism homeodynamics is important; if not spermatogenesis will be arrested. Glycogen plays essential role in testis. Sertoli cells use extracellular-glucose through GLUT-5 the specific glucose transporters.[282] GLUT-5 facilitates the transporting of glucose into sperm cell, which is used as substrate in the production of energy.[283] Presence of energetic-reservoir in glycogen form will sustain the energy and therefore viability of sperm from ejaculation to fecundation and increases the reproduction potential. [263] Significant decrease in the glucose level and significant increase ($P<0.001$) in the motility of the sperm after treatment may state that the drug may act on glycogen phosphohexose pathway, by which frutose may arises from glucose which is needed for sperm motility.[262] *Tribulus terrestris; Mucuna prurita; Asphaltum punjabinum; Cuminum cyminum; Maerua arenaria; Cinnamomum tamala; Vitis vinifera; Bombax ceiba; Costus speciosus; Phoenix dactilifera; Cyperus rotundus; Coscinium fenestratum are the ingredients present in CKC with antihypergycemic activity* [as refered in drug review] and due to which the glucose level might be decreased significantly after treatment and utilized for energy.

Cholesterol [**Fourth precursor in the formation of sperm]**

Increased BMI is related to poor quality of semen, decreased sperm count and normal-sperm motility.[259] Significant decrease in cholesterol level after treatment (CKC) may be due to anti hyperlipidemic effect of *Cuminum cyminum; Glycyrrhiza glabra; Cinnamomum tamala; Cyperus rotundus; Phoenix dactlifera; Costus specious; Coscinium fenestratum; Mucuna pruriens; Myristica fragrans; Cumin cyminum; Asphaltum punjabinum; Syzgium aromaticum* [as refered in drug review] and additionally due to the astringent taste present in *CKC* which reduces cholesterol level.[39] Thus the drug balances the cholesterol level the fourth body constituent and fourth precursor in the formation of sperm.

Bone [Fifth precursor in sperm formation]

The significant increase in the testosterone [steroid harmone] reported after treatment may proceed on osteoblast/osteoclast and modify the bone formation.

Bone marrow [Sixth precursor in the formation of sperm]

Element iron is present in *CKC*. *Asphaltum punjabinum* the ingredient present in *CKC* helps in the absorption of iron into the body and makes it bioavailable to bone marrow stem cells and additionally *Phoenix dactilifera* have stimulatory effect on the bone marrow tissue [185,158]

Semen/Sperm

In the present study spermatogenesis is enhanced which is evident from the significant increase in sperm parameters after the treatment which may be due to the presence of steroid, amino acids, iron, calcium, magnesium, zinc and copper with respect to sperm production, maturation and motility.On the basis of observation it may be concluded the drug nourishes the body constituents and improves the sperm parameters [count, motility and normal form of sperms].

8. SUMMARYAND CONCLUSION

- The study was conducted to explore and establish the safety and efficacy of the siddha herbo-mineral formulation *Chandrakanthi chooranam* in the treatment of oligospermia through clinical and preclinical studies and to ensure the quality of the drug through analytical studies.

- Heavy metals and aflatoxins were found to be below detection level. Bacterial and fungal count [microbial contamination] was found to be within the prescribed limits. Specific pathogens and pesticide residues were found to be absent. The results propose that the prepared drug study is of standard quality.

- Acute toxicity study of the drug *Chandrakanthi chooranam* revealed that it didn't produce any signs of toxicity sign and is well tolerated up to 10 times [10.8gm/kg b.wt] the therapeutic dose in tested wistar rats. Long term toxicity study for 90 days in wistar rats revealed that the no-observed-adverse-effect-level of *Chandrakanthi chooranam* was found to be 1 TD and 3TD dose level with respect to animal survival, haemotological, biochemical and histopathological findings and low adverse effect was found to be in 5TD dose level with reference to significant increase in total protein and significant decrease in the organ weight of heart and lungs.

- The findings of this study bring forth the spermatogenic activity of *Chandrakanthi chooranam* and showed protctive effect on sperm count, motility, viability and normal forms of sperm in rats treated with ethanol. The combined androgenic and antioxidant activity may be the cause for its positive results on spermatogenesis.

- Pilot study in clinical was found to be feasible, no safety issues occurred and the trial drug showed significant increase in the sperm parameters. The main clinical trial showed significant increase in sperm count, motility and morphology and it may be due to the presence of the steroidal constituent of *Chandrakanthi chooranam* and its meiosis inducing effect during the Spermatogenesis. This proposition is further comparable with the significant increase in testosterone level which stimulate spermatogenesis.

- The Trial conclude and set forth the drug *Chandrakanthi chooranam* was proved to be safety and efficacy in preclincal studies and clinical trial and the outcome obtained present it as standard treatment for patients with oligospermia by improving sperm parameters.

9. RECOMMENDATION

- The study states *Chandrakanthi chooranam* had showed significant increase in sperm parameters and testosterone level and it is recommended for the treatment of oligospermia leading to infertility

- Animal experimentation which requires further research works to elucidate the antioxidant activity and effect of the trial drug on antisperm antibodies.

- It is recommended that Phase III -Therapeutic Confirmatory Trials should be done in a larger number of patients in comparison with a standard/ placebo drugs to validate the safety and efficacy that is established in the present Phase II trial.

- The limitation of the present study comprise the asessment of the fertility potential of *Chandrakanti chooranam* in oligospermic patients and hence the drug should be further directed to asess the fertility potential.

- The drug improves the sperm quality and is recommended to be harmonized with assisted fertility treatments. The oligozoospermic patients should be treated with *Chandrakanti chooranam* to improve sperm quality before performing retrieval of sperm.

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CASE SHEET PROFORMA
National Institute of Siddha, Chennai, Tamilnadu, India
Safety and Efficacy of *Chandrakanthi Choornam* in Oligospermia –
A Preclinical and Clinical study
FORM-I - SCREENING AND SELECTION PROFORMA

1. Name:

2. S.I. No		3.O.P.D No	
4. Age:		5. Sex	

6. Address & Phone No

SELECTION CRITERIA

Inclusion criteria

Male infertile patients with age between 21-45 years
Marriage history for >1 year
Sperm count 1-15 million/ml [below one million is excluded]
Patients with normal liver & renal function test
Willing to give specimen of semen before & at the end of the clinical trial
Informed patients giving written consent

Exclusion criteria

Azoospermia-complete absence of sperm in the ejaculate
Aspermia-complete lack of semen
Necrospermia- spermatozoa in semen are dead
Clinical diagnosis of varicocele & hydrocele
History of undescended testis
Inguinal hernia on physical examination
Male accessory gland infection
History of DM, Hypertension and Cardiac Disease
Any recent medical or surgical illness
Underwent treatment for promoting spermatogenetic fertility in last 3 months
Other systemic disease requiring specific therapies
Known Thyroid disease
Past history of renal, hepatic or any other chronic illness in the patient

Patient is included in the Trial YES / NO

Date: **Signature of the Investigator:**

National Institute of Siddha, Chennai, Tamilnadu, India
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FORM-II - HISTORY PROFORMA

1. Name:

2. S.I. No [ ] 3. O.P.D No [ ]

4. Age: [ ] 5. Sex [ ]

6. Permanent Address & Phone No

7. Education : Illiterate / Primary / Secondary / Graduate / Post graduate

8. Occupation : Labour / Intelectual / Sedentary

Nature of Work: Physical / Intelectual / Chemical or radiation / Thermal or night shift

9.Socio economical status: L / M / U

10. COMPLAINTS AND DURATION :

No issues since [ ] years

Primary Infertility [ ] Secondary Infertility [ ]

11. HISTORY OF PRESENT ILLNESS

12. HISTORY OF PREVIOUS ILLNESS

Mumps / orchitis / Prostatitis / STD / Hypertension / Cardiac disease / DM /

Scrotalinjuries / TB / Filariasis / others

13. SURGICAL INTERVENTION

Hydrocele / Varicocele / Blockage of Vas / Vasectomy / Hernia /

Obstruction of ejaculatory duct / Trauma

14. PERSONAL HISTORY

Diet	Veg / Mixed
Food habits	Irregular pattern / starvation / poor eating / over eating / incompatabile food / unwholesome diet / normal
Sleep	Sound / disturbed / delayed
Exercise	Heavy / Moderate / less / no
Addiction	Smoking / Alcohol / Tobacco / Smoking & Alcohol / No addiction
Undergarments	Synthetic Tight / Synthetic Loose / Cotton Tight / Cotton Loose
Bath	Hot water / Normal water

15. DRUG HISTORY

Addiction / Steroids / Antidepressant / Opiod / Chemotherpeutic agents /

Cyclophosphamide / others

16. MARITAL HISTORY

Duration of Marriage ☐ years Consanguineous - Yes / No

17. SEXUAL HISTORY

Sexual desire: Lack / Normal / Excess

Coitus : Pain / Weakness / Burning / normal

Pervertion Yes / No

Mastrubation - Yes / No

18. PSYCHOLOGICAL HISTORY

Normal / Depressed / Anxious / Stress / Anger / Fear

19. FAMILY HISTORY

Congenital anomalies / Crypto-orchidism / Hypospadias / issueless / delayed conception

History of conception (Wife's) - Abortion ☐ Miscarriage ☐

Date : **Signature of the investigator**

National Institute of Siddha, Chennai, Tamilnadu, India
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FORM-III – CLINICAL ASSESSSMENT PROFORMA

1.Name:

2. S.I. No		3.OPD No	
4. Age:		5. Sex	

A] **SIDDHA ASPECT**

I] Nilam (Land): Kurinji / Mulai / Maruthuam / Neithal / Palai

II] Paruvakalam (Season) : Kaar / Koothir / Munpani / Pinpani / Elavenil / Muthuvenil

III] Thega Nilai (Bio Type) : Vatham / Pitham / Kabam / Thontham

IV] Gunam (Character) : Sathuvam / Rasatham / Thamasam

V] Vanmai (Bulit) : Iyalbu (Normal) / Valivu (Heavy) / Melivu (Lean)

VI] Poripulangal (Sensory Organs)

Parameters	Assessment
Kai (Upper limb)	Normal / Affected
Kaal (Lower limb)	Normal / Affected
Vai (Buccal Cavity)	Normal / Affected
Eruvai (Excretory organs	Normal / Affected
Karuvai (Reproductive organ)	Normal / Affected

VII]Kanmendriyam (Motor Organs)

Parameters	Assessmet
Mei (Skin)	Normal / Affected
Vai (Buccal cavity)	Normal / Affected
Kann (Eye)	Normal / Affected
Mooku(Nose)	Normal / Affected
Sevi (Ear)	Normal / Affected

VIII Kosangal (Sheath)

Parameters	Assessment
Annamayakosam	Normal / Affected
Pranamayakosam	Normal / Affected
Manomayakosam	Normal / Affected
Vignanamayakosam	Normal / Affected
Anandhamayakosam	Normal / Affected

IX] Seven Thathus (Seven somatic components)

Parameters	0th Day	91st Day
Saaram	Normal / Affected	Normal /Affected
Senneer	Normal / Affected	Normal /Affected
Oon	Normal / Affected	Normal /Affected
Kozhupu	Normal / Affected	Normal /Affected
Enbu	Normal / Affected	Normal /Affected
Moolai	Normal / Affected	Normal /Affected
Sukkilam	Normal / Affected	Normal /Affected

X] Mukkutram : [Affection of Three Humors]

Parameters	0th Day	91st Day
VATHAM		
Pranan	Normal / Affected	Normal /Affected
Abanan	Normal / Affected	Normal /Affected
Uthanan	Normal / Affected	Normal /Affected
Viyanan	Normal / Affected	Normal /Affected
Samanan	Normal / Affected	Normal /Affected
Koorman	Normal / Affected	Normal /Affected
Naagan	Normal / Affected	Normal /Affected
Kirukaran	Normal / Affected	Normal /Affected
Devathathan	Normal / Affected	Normal /Affected
Dhanjeyan	Normal / Affected	Normal /Affected
PITHAM		
Aakkanal	Normal / Affected	Normal /Affected
Vanna eri	Normal / Affected	Normal /Affected
Alosakam	Normal / Affected	Normal /Affected
Prasagam	Normal / Affected	Normal /Affected
Aarralanki	Normal / Affected	Normal /Affected
KABAM		
Avalampakam	Normal / Affected	Normal /Affected
Kilethegam	Normal / Affected	Normal /Affected
Pothakam	Normal / Affected	Normal /Affected
Tharpakam	Normal / Affected	Normal /Affected
Santhikam	Normal / Affected	Normal /Affected

XI] Envagai Thervu : [Eight- fold examination]

S.NO	Envagai Thervu	Parameters	0th Day	91st Day
1.	Naa (Tongue)	Thanmai (Character)	Normal / Affected	Normal / Affected
		Niram (Colour)	Normal / Affected	Normal / Affected
		Suvai (Taste)	Normal / Affected	Normal / Affected
2.	Niram (Complexion)	Examination of colour	Normal / Affected	Normal / Affected
3.	Mozhi (Voice)	Thanmai (Character)	Normal / Affected	Normal / Affected
4.	Vizhi (Eye)	Niram (Colour)	Normal / Affected	Normal / Affected
		Thanmai (Character)	Normal / Affected	Normal / Affected
5.	Malam (Stools)	Thanmai (Character)	Normal / Affected	Normal / Affected
		Niram (Colour)	Normal / Affected	Normal / Affected
		Vemmai (Warmth)	Present / Absent	Present / Absent
6.	Sparisam (Palpatory perception)	Veppam (Warmth)	Normal / Affected	Normal / Affected
		Thoduvali (Pain)	Present / Absent	Present / Absent
7.---------.	Naadi (Pulse)	Thanmai (Pattern)		
8.	Moothiram (Urine) i)Neerkuri (Urine examination)	Niram (Colour)	Normal / Affected	Normal / Affected
		Manam (Odour)	Normal / Affected	Normal / Affected
		Nurai (Froth)	Present / Absent	Present / Absent
		Edai (Specific gravity)	Normal / Affected	Normal / Affected
		Kalapu (Deposits)	Present / Absent	Present / Absent
		Enjal (Volume)	Normal / Affected	Normal / Affected
	ii)Neikuri (Oil on urine sign)			

XII] Status of Panchagni (Basal metabolic heat)

Parameters	0^{th} Day	91^{st} Day
Samagni	Normal / Affected	Normal /Affected
Mandagni	Normal / Affected	Normal /Affected
Teekshagni	Normal / Affected	Normal /Affected
Vishamagni	Normal / Affected	Normal /Affected

XIII] Food Affinity:

Taste: Innipu(sweet)/ Pulipu(sour)/ Karpu(acrid)/ Kaipu(bitter)

/ Uppu(salt)/ Thuvarpu(astringent)

Hot / Cold

B] MODERN ASPECT

I] General Examination

Height	**Respiratory rate**	**Cyanosis**
Weight	**Blood pressure**	**Clubbing**
BMI	**Temperature**	**Lymphadenopathy**
Pulse rate	**Pallor**	**Pedal edema**
Heart rate	**Jaundice**	**Jugular Vein Pulsation**

II] Systemic Examination

1. **Respiratory system**
2. **Cardio vascular system**
3. **Digestive system**
4. **Nervous system**

5.Male genital system

S.No	Parameters		Assessment
1.	**Pubic hair**	**Distribution**	No / light / dark / small / extends to thigh & umblicus
2.	**Inguinal lymph node**	**Palpation**	Palpable / Non palpable
3.	**Penis**	**Skin**	Normal / Redness / Swelling / Scar / Ulcer
		Body /shaft :	Normal / Curved / shrunken / wound
		Prepuce	Normal / Phimosis / Paraphimosis / Circumcessed
		Glans	Normal / Ulcer / Scars / Balanitis / Balanoasthitis
		Urethral meatus	Normal / Hypospadis / Epispadis / Discharge
4.	**Scrotum**	**Skin**	Normal / less fold / Nodules / Redness / Ulceration
		Pigmentation	Normal / Hyper / Hypo
		Sac	Normal / Sagging / Hydrocele
		Hernia	Right – direct / Indirect ; Left – direct / Indirect
		Cremastic reflex	Present / Absent
5.	**Testis**	**Position**	Normal / Retracted / Criptoorchid
		Surface	Smooth / Nodular
		Consistency	Firm / Soft
		Borders	Regular / Irregular
6.	**Epididymis**	**Palpation**	Tender / Nontender
7.	**Spermatic cord**	**Palpation**	Normal / Thickened / Varicocele
8.	**Vasa**	**Palpation**	Tender / Nontender
9.	**Rectal examination (Prostate gland)**	**Consistency**	Normal / Hard / Boggy
		Palpation	Tender / Nontender
		Surface	Smooth / Nodular / Other impression

III] Clinical Symptoms

Clinical Symptoms	0^{th} day	16^{th} day	31^{st} day	46^{st} day	61^{th} day	76^{th} day	91^{st} day
Premature ejaculation	Present / Absent	Present /Absent	Present / Absent	Present / Absent	Present / Absent	Present Absent	Present /Absent
Nocturnal emission	Present / Absent	Present / Absent	Present / Absent	Present / Absent	Present / Absent	Present / Absent	Present /Absent
Erectile dysfunction	Present / Absent	Present / Absent	Present / Absent	Present / Absent	Present / Absent	Present / Absent	Present /Absent

Date : **Signature of the investigator**

National Institute of Siddha, Chennai, Tamilnadu, India
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FORM-IV – LABORATORY PARAMETER

1. Name:

2. S.I. No [ ] 3.OPD No [ ]

4. Age: [ ] 5. Sex [ ]

5.Date of Assessment [ ]

Blood Investigation		**0th Day**	**91st Day**	**121st Day**	**151st Day**	**181st Day**
HB (gms %)						
RBC (milli/cu.mm)						
ESR (mm)	1/2hr					
	1hr					
T.WBC (cu.mm)						
Differential count (%)	Polymorphs					
	Lymphocytes					
	Monocytes					
	Esnophils					
	Basophils					
Blood Glucose (mg/dl) (R)						
Lipid Profile (mg/dl)	Cholesterol					
	HDL					
	LDL					
	VLDL					
	TGL					
RFT (mg/dl)	Blood urea					
	Serum creatinine					
	Serum uric acid					
LFT (mg/dl)	Total bilirubin					
	Direct bilirubin					
	Indirect bilirubin					
	Serum total protein					
	Serum Albumin					
	Serum globulin					
	Fibrinogen (g/dl)					
	Serum calcium					
	Serum phosphorus					
	SGOT (IU/L)					
	SGPT (IU/L)					
	Alkaline phosphatase (IU/L)					
VDRL						
HBsAg						

Urine Investigation	**0th Day**	**91st Day**	**121st Day**	**151st Day**	**181st Day**
Albumin					
Random sugar					
Deposits					
Bile salts					
Bile pigments					
Urobilinogen					

Semen Analysis	**0th Day**	**91st Day**	**121st Day**	**151st Day**	**181st Day**
Collection					
Abstinence Period					
Method of collection					
Time of collection					
Time of examination					
Colour					
Appearance					
Volume (ml)					
Liquefication time (mt)					
Viscosity					
Pus cells					
Sperm count (million/ml)					
Motility(%) Total Motility					
Progressive Motility					
Immotile					
Morphology (%) Normal					
Abnormal - Head Defect					
Tail Defect					

Harmone Analysis	**0th Day**	**91st Day**	**181st Day**
TSH			
LH			
Testosterone			

Blood group

ECG

Others:

Date : **Signature of the Investigator**

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FORM-V – ADVERSE REACTION FORM

1. Name:

2. S.I. No | 3.OPD No

4. Age: | 5. Sex

Sl.no	Particulars	Details
1	Study site	
2	Brief description of the event	
3	Date of onset	
4	Time of onset	
5	Date of administration of 1st dose of study drug	
6	Time of administration of 1st dose of study drug	
7	Date of administration of last dose of study drug	
8	Time of administration of last dose of study drug	
9	Severity of the AE	
10	Did the subject hospitalized	
11	Relationship to the study drug	

To be filled by the investigator

12	Did the event require to stop the study drug	
13	Out come of the event	
14	Date and time of report	
15	Signature of the investigator	

The Adverse Effects will be intimated to the Institution Ethical Committee within 48 hours of time.

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FORM-V A– WITHDRAWAL FORM

1. Name:

2. S.I. No | 3.OPD No

4. Age: | 5. Sex

Date of Trial Commencement:

Date of Withdrawal from Trial:

Reasons for Withdrawal:

Long absence at reporting	Yes / No
Irregular treatment	Yes / No
Shift of locality	Yes / No
Increase in severity of symptoms	Yes / No
Development of severe adverse drug reactions	Yes / No

Date: Signature of the Investigator

Signature of the Guide

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FORM-VI –DRUG COMPLIANCE FORM

Name	S.I.No	OPD No

Name Of The Drug :CHANDRAKANTHI CHOORNAM
Drugs issued: (Grams)
Drugs returned: (Grams)

Day	Date	Time
1		
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3		
4		
5		
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Day	Date	Time
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Signature of the Patient

Safety and Efficacy of *Chandrakanthi Choornam* in Oligospermia – A Preclinical and Clinical study

FORM-VI A – DIETARY ADVICE FORM

I] Pathiyam (Diet and Behaviors to Follow)

Diet:

Greens of Drumstick, Climbing brinjal, Spinach and Amaranthus tritis
Goat meat; Emperor and Eel fish
Plantain flower, Drumstick
Mango fruit, Black grapes, Black plum and Pomegranate
Cow's Milk
Cashew, Almond and Walnut

Behaviour:

Patients were adviced to take oil bath twice in a week
Avoid intercourse on the day of the oil bath

II] Apathiyam (Diet and Behaviors to Avoid)

Diet:

Horse gram
Mango, Bitter guord
Sesban leaves

Behaviour:

Avoid intercourse in day time and during digestion of the food

Other Advices

Tobacco, Smoking, Alcohol, Drug abuse
Hot baths
Strenuous activities
Occupation in hot environment,
Wear loose under wear
Control obesity

Safety and Efficacy of *Chandrakanthi Choornam* in Oligospermia – A Preclinical and Clinical study

FORM-VII – PATIENTS INFORMATION SHEET

You are asked to participate in this study. Therefore, the following information is given for better understanding of how the research study is being done and to know about your rights to decide to participate/ not to participate in this study.

Purpose of the study: To provide effective treatment in the oligospermia through siddha formulation.

Total number of participants and duration of the study: 40 participants and 90 Days

Treatment procedure: For every 15 days once the participants should come to OPD for consultation, and collection of medicine. Blood, urine and semen test will be conducted before and after and during the follow up period**.**

Laboratory investigations to be done: Initially screening of the semen, blood & urine will be done to know the eligibility criteria for participating in the study**.** You will be involved to undergo for semen test in the beginning, at the end of the treatment period and follow up period to assess the control of disease. Siddha parameters like Naadi, neikuri and neerkuri will be done on 1st day and also in end of the trial.

Benefits to the participants: Medicines, semen, urine, blood test will be carried out at free of cost during the study period. It is hoped that the treatment you receive will help to increase the sperm count.

Withdrawal criteria: If you are uncomfortable in any situation during the study period, you have the right to withdraw from the study at any time. You will be provided regular health service.

Adverse effects: In case of any adverse effects occur during the study period, it should be informed to investigator. Necessary arrangements will be made for immediate treatment.

Benefit for the society: Results of this study may be useful to decide oligospermia therapy with siddha formulations for the larger population.

Remuneration: You will not be paid any remuneration for participating in this study.

Confidentiality: The information provided by you will be kept in strict confidence. Under no circumstance shall I reveal the identity of the participant or their family to anyone.

Publications of the research results: The information that I collect shall be used for approved research purposes with ID number.

Contact person: Whenever you have any doubt regarding disease/medicine/treatment, you have rights to ask any questions to the investigator.

Dr.Akila.M.D(S), PhD Scholar, Department of Maruthuvam,
National institute ofSiddha, Chennai-47; Cell no: 9444645833
National Institute of Siddha, Chennai, Tamilnadu, India

Safety and Efficacy of *Chandrakanthi Choornam* in Oligospermia – A Preclinical and Clinical study

FORM-VII A – CONSENT FORM

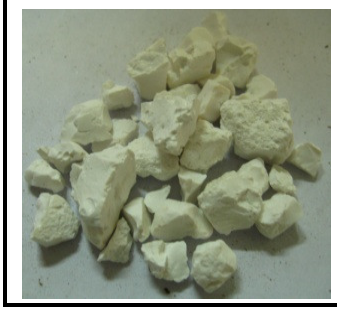
I_____S/o_____

Study Participant ID No:_____ have received the verbal information regarding the above study. The above information have been read by me / read to me, and have been explained to me by the investigator. I was informed that I may withdraw myself from the study at any time without any reason and I ensure that I will take medicines as recommended by the investigator. I agree to use my research data as described in the informed consent form. Having understood the same, I hereby give my consent to participate. I affixing my signature/ left thumb impression to indicate my consent and willingness to cooperate in this research study.

	Name	**Signature**
Participant		
Investigator	**Dr.B.Akila**	
Witness Relation ship		

Figure 5.1.1: Ingredients present in Chandrakanthi choornam

Bambusa aurundinaceae



Myristica fragrans

Cyperus rotundus

Ilicium verum

Coscinium fenestratum

Adhatoda vasica

Lawsonia inermis

Alternanthera sessilis

Asphaltum punjabinum

Figure 5.1.2: Prepartion of Gomutra Silasathu Parpam

5.1.2A Layer formation **5.1.2B Pellets**

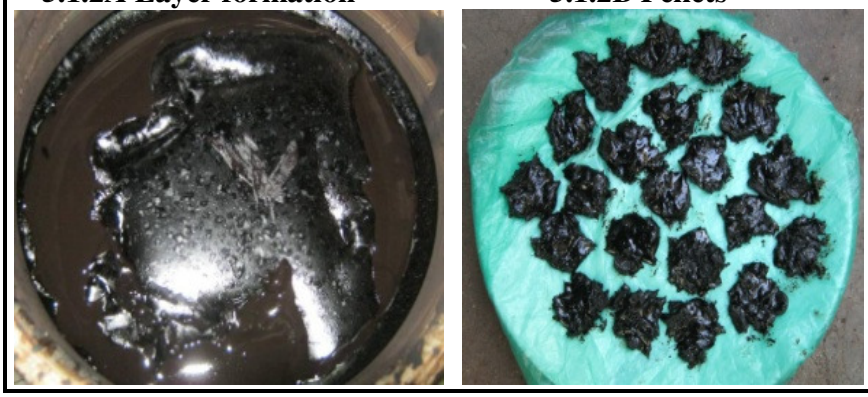
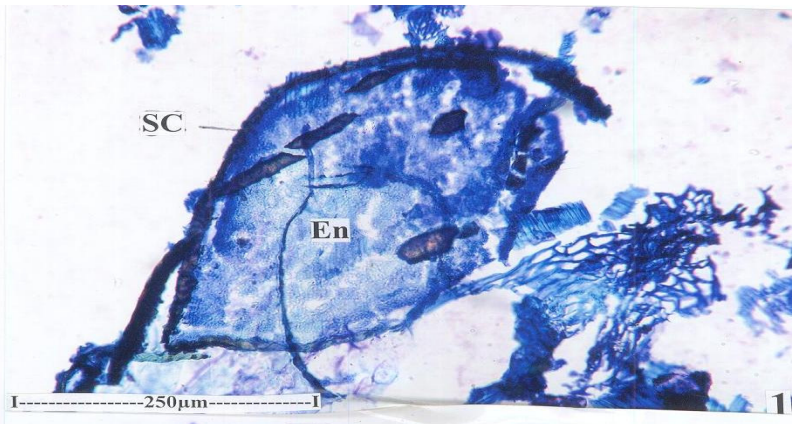


Figure 5.1.3 ***Chandrakanthi choornam***

Figure 6.1 (a) : *Alternanthera sessilis* flower with seed

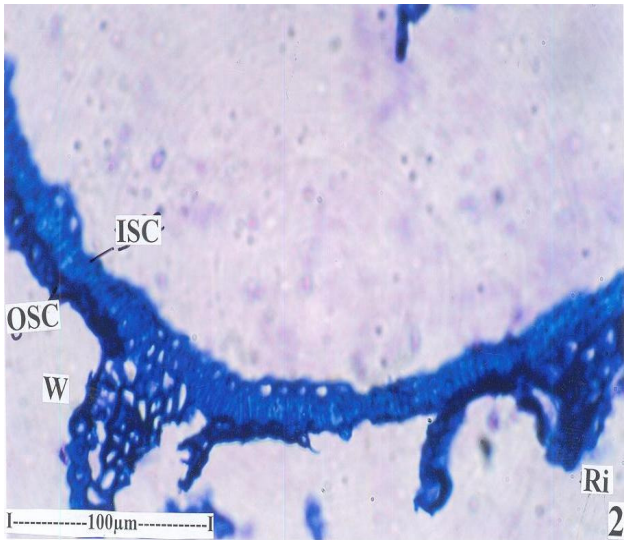
Figure 6.1 (b): External feature of *Alternanthera sessilis* seeds

Figure 6.2: LS of *Alternanthera sessilis* seed (entire view -16x



(En- Endosperm; Sc- Seed coat)

Figure 6.3: *Alternanthera sessilis* seed coat with sclerotic testa and outer outgrowing wing - 40x



(ISC:Innerseedcoat;OSC:outerseedcoat;W:Wing)

Figure 6.4:TS of *Alternanthera sessilis* seed (10x)

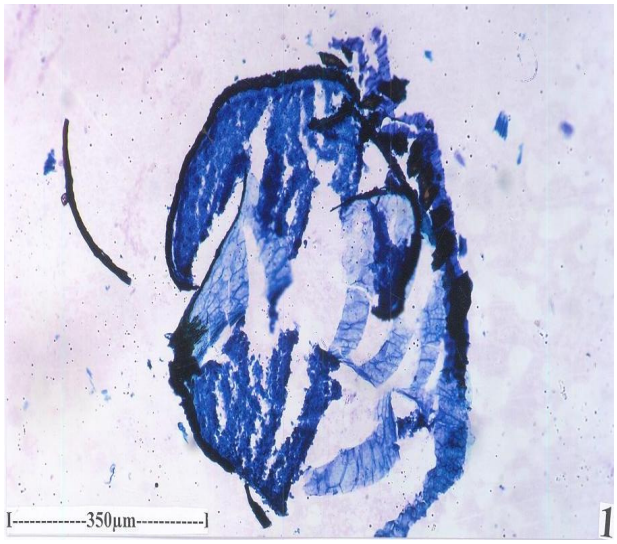
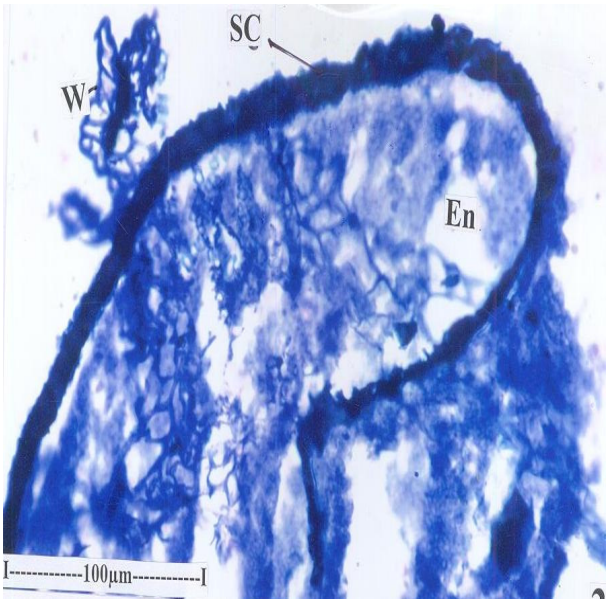
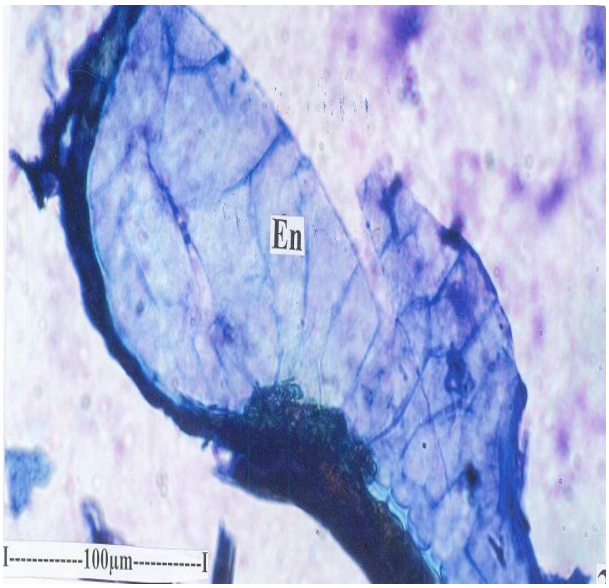


Figure 6.5: *A. sessilis* seed coat with outer wing - 40x



(En- Endosperm; Sc- Seed coat)

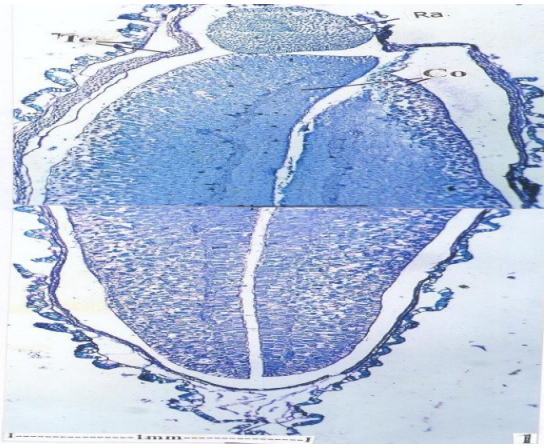
Figure 6.6: Cellular endosperm of *A.sessilis* seed – 40x



(En- Endosperm)

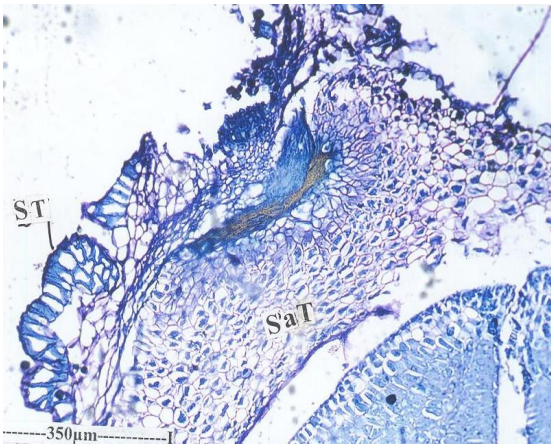
Figure 6.7: *Adhatoda vasica* seeds external features showing median ridge and rugose surface (1x, 5x)

Figure 6.8: Vertical tangential longitudinal section of *Adhatoda vasica* seed reconstructed -4x



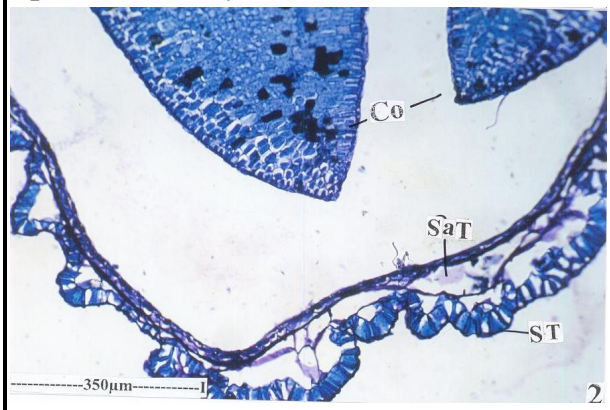
(CO: Cotyledons, RA: Radicle, TE: Testa)

Figure 6.9: LS of *Adhatoda vasica* seed upper chalazal position-10x



(SAT: Sarcotesta, ST: Sclerotesta)

Figure 6.10:Lower portion of the seed shows a part of the cotyledons and seed coat-10x



(CO: Cotyledons)

Figure 6.11: Sectional view of the *Adhatoda vasica* seed coat 10x

(CO: Cotyledon, ST: Sclerotesta)

Figure 6.12:A portion of the cotyledons-40x

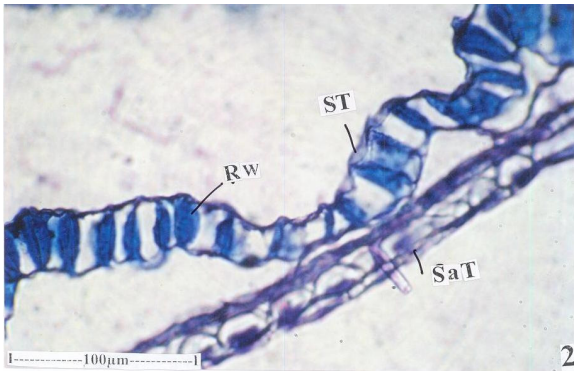
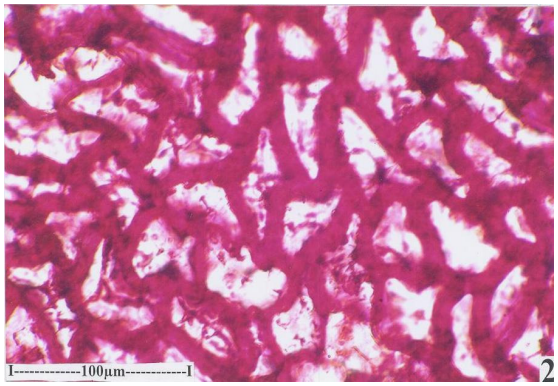


Figure 6.13: *A.vasica* seed coat epidermis sclerotesta in surface view-16x

(SAT: Sarcotesta; RW: Radial wall-thick and lignified)

Figure 6.14:Epidermal cells-enlarged-40x



CW: Cell wall

Figure 6.15: Inner part of the seeds coat sarco-testa in surface view -40x

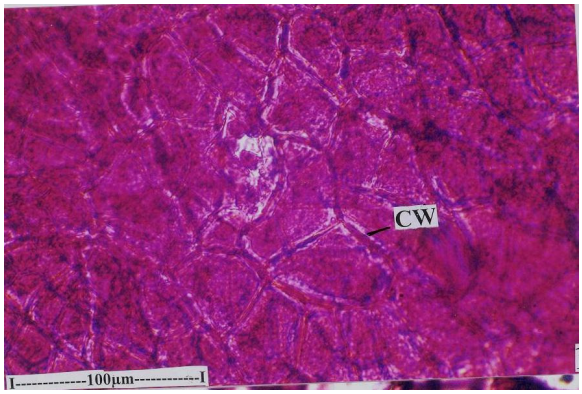
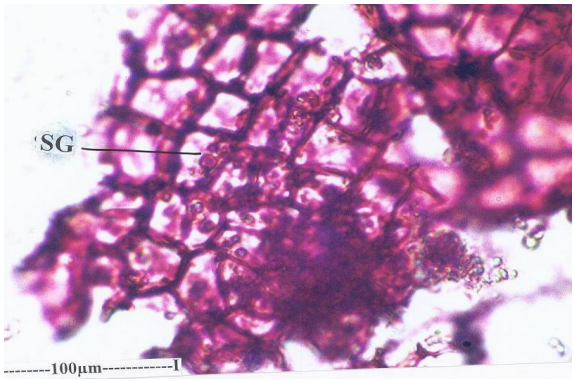


Figure 6.16:cells of cotyledons with starch grains-40x



(SG: Starch grain)

Figure 6.17: Isolated cotyledon-40x

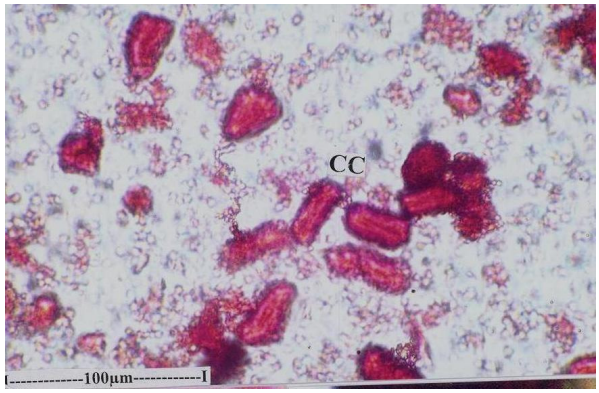


Figure 6.18: Oil-bodies-40x

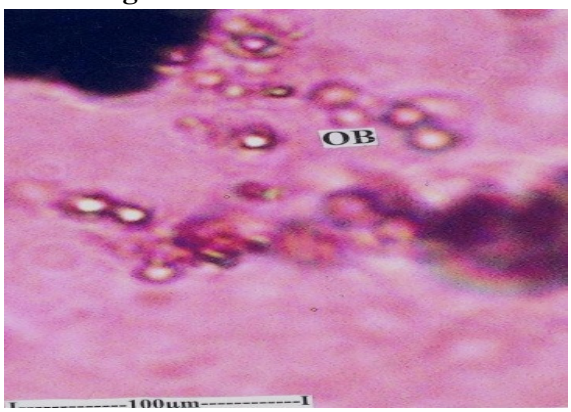
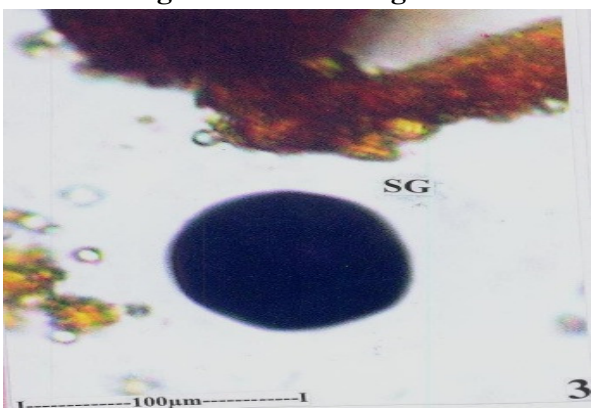
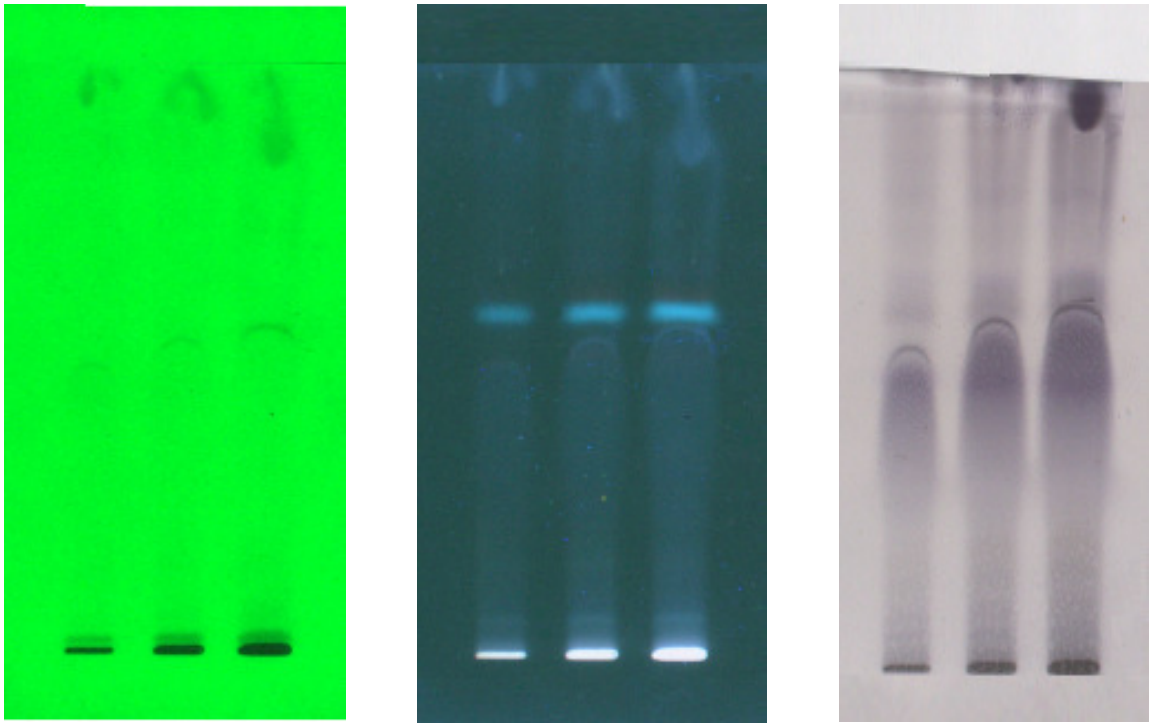


Figure 6.19. Starch grain-40x





1 2 3 1 2 3 1 2 3

A.Under UV 254 nm **B.UV 366 nm** **C.After Derivatization**

Figure 6.20: TLC profile of chloroform extract of *A. vasica* seed
Track 1. 5µl; Track 2. 10 µl; Track 3. 15 µl.

Figure 6.21A: HPTLC Finger print profile $CHCL_3$extract of *A.vasica* seed at uv 254 nm

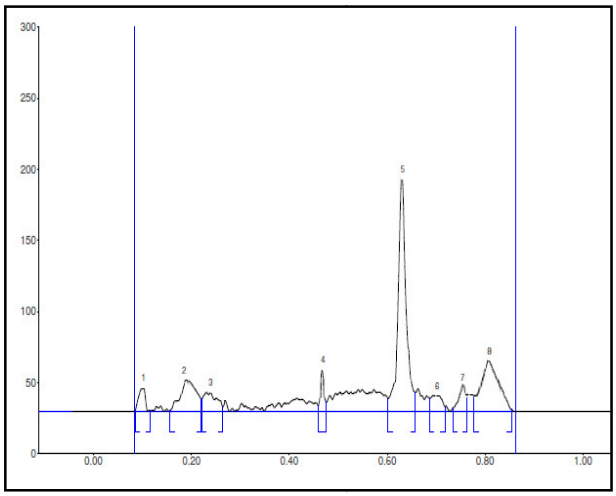


Figure 6.21B: 3D of Chromatogram of $CHCL_3$ extract of *A.vasica* seed at uv 254 nm

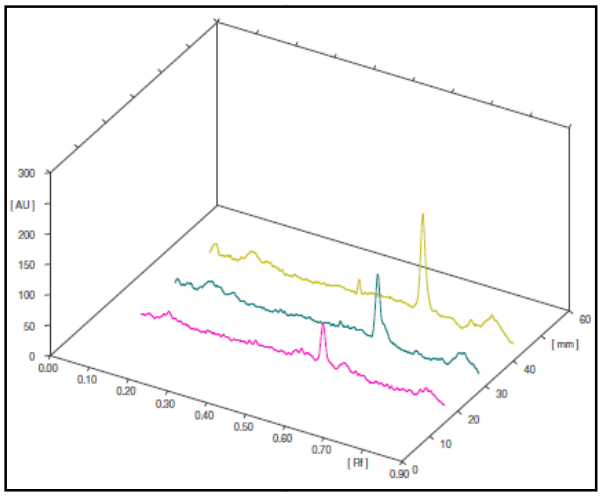


Figure 6.22A: HPTLC Finger Print profile of $CHCL_3$extract of A. vasica seed at uv 366 nm

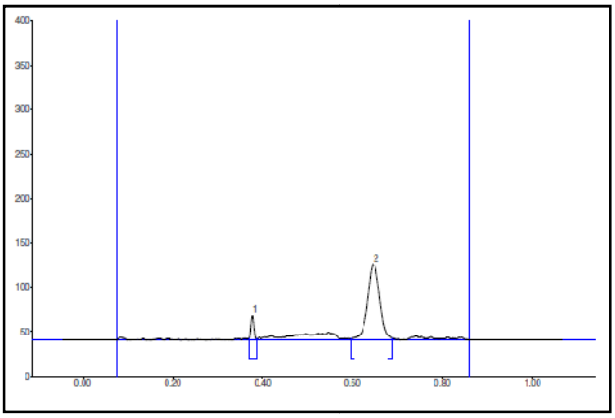


Figure 6.22B: 3D Chromatogram of $CHCL_3$ extract of a. vasica seed at uv 366 nm

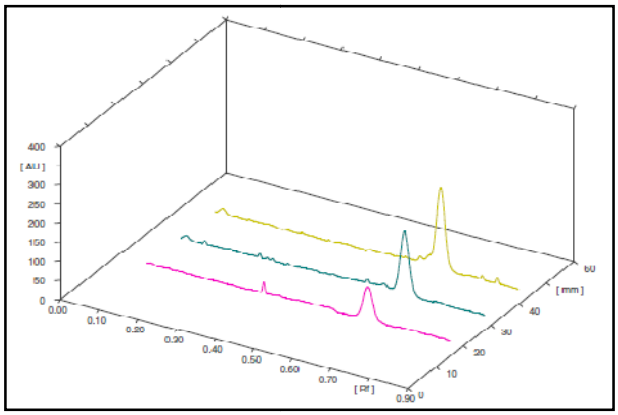


Figure 6.23A: HPTLC Finger print profile of $CHCL_3$ extract of *A.vasica* seed at 540 nm

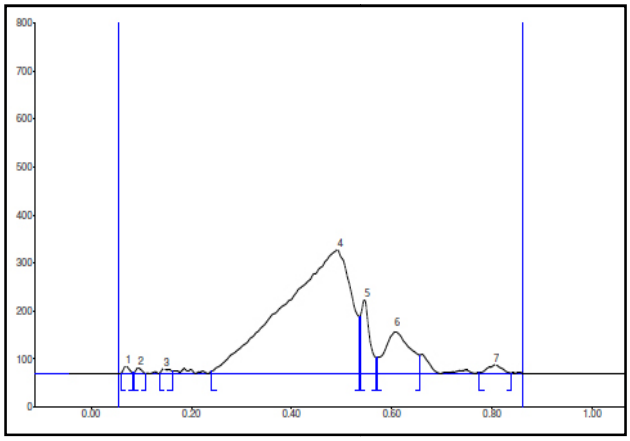


Figure 6.23B:3D Chromatogram of all tracks of $CHCL_3$ extract of *A.vasica* seed at 540 nm

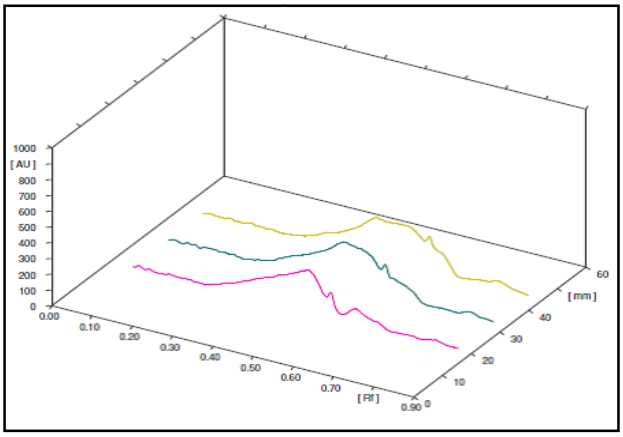
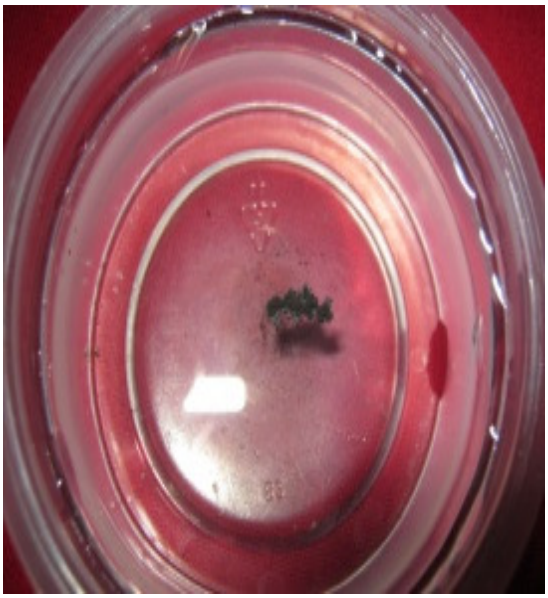
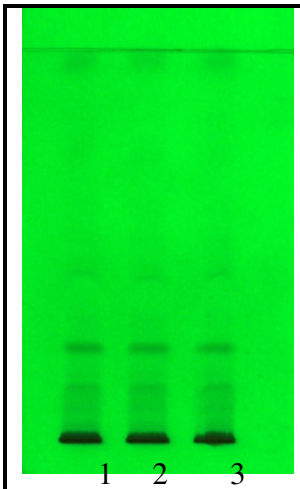


Figure 6.24 Siddha specification for Gomutra Silasathu Parpam

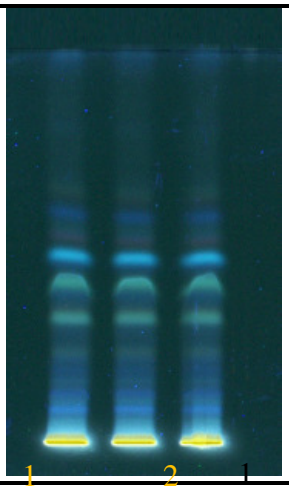
Floats on water



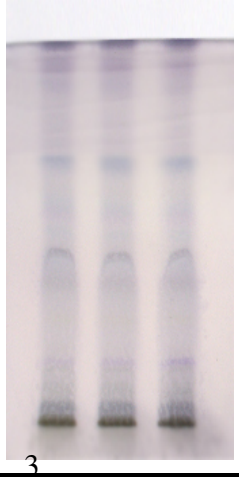
Lines of the thumb finger



A.Under UV254 nm



B.UV 366 nm



C.Derivatized

Figure 6.25: TLC profile of $CHCl_3$ extract of Chandrakanthi chooranam. Track 1. 7µl; Track 2. 10 µl; Track 3. 12 µl.

Figure 6.26: HPTLC finger print of $CHCl_3$ extract of CKCat UV 254 nm

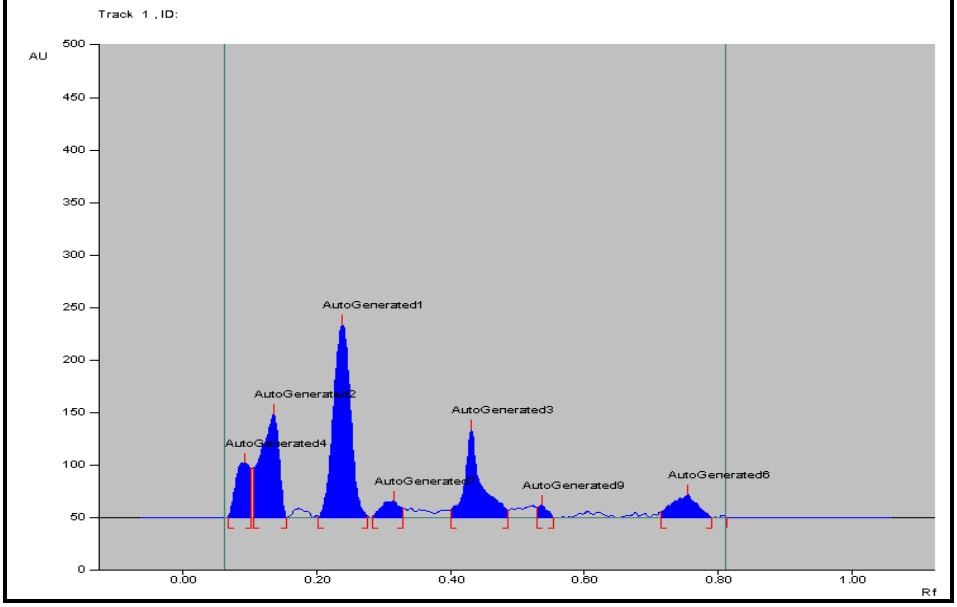


Figure 6.27. HPTLC finger print of $CHCl_3$ extract of *CKC* at UV 366 nm

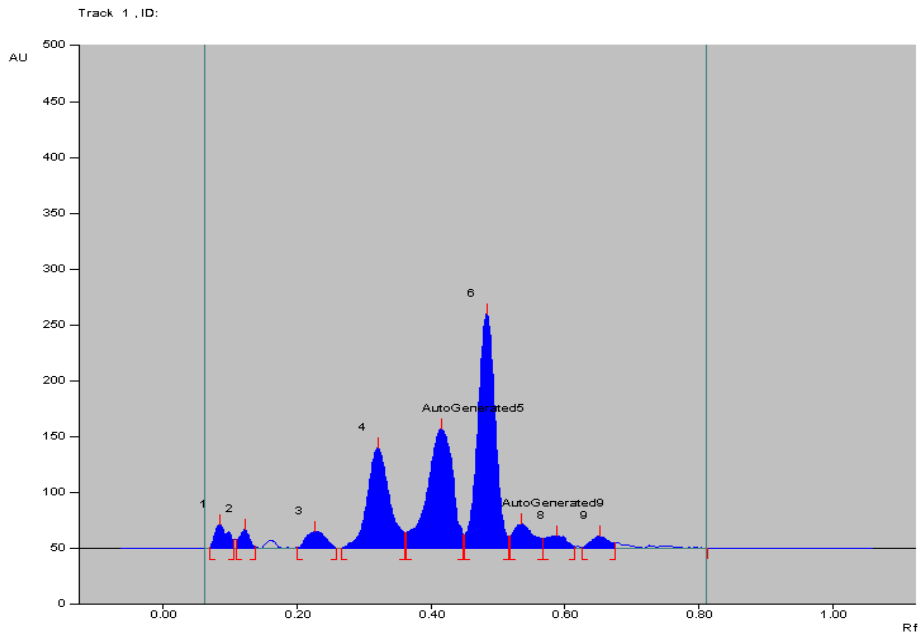


Figure 6.28 HPTLC finger printing of $CHCl_3$ extract of *CKC* at UV 540 nm

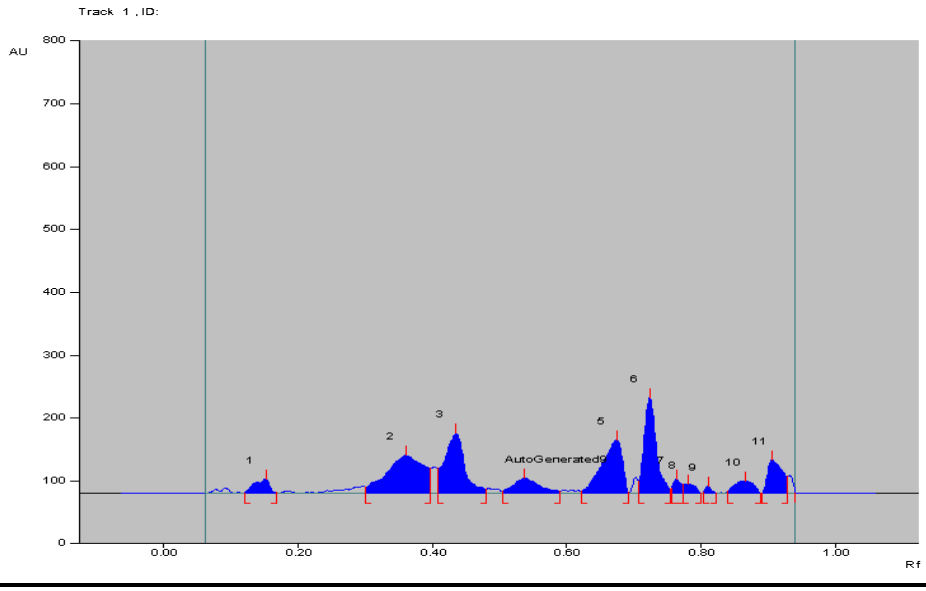


Figure 6.29. TGA spectra of *Chandrakanthi choornam*

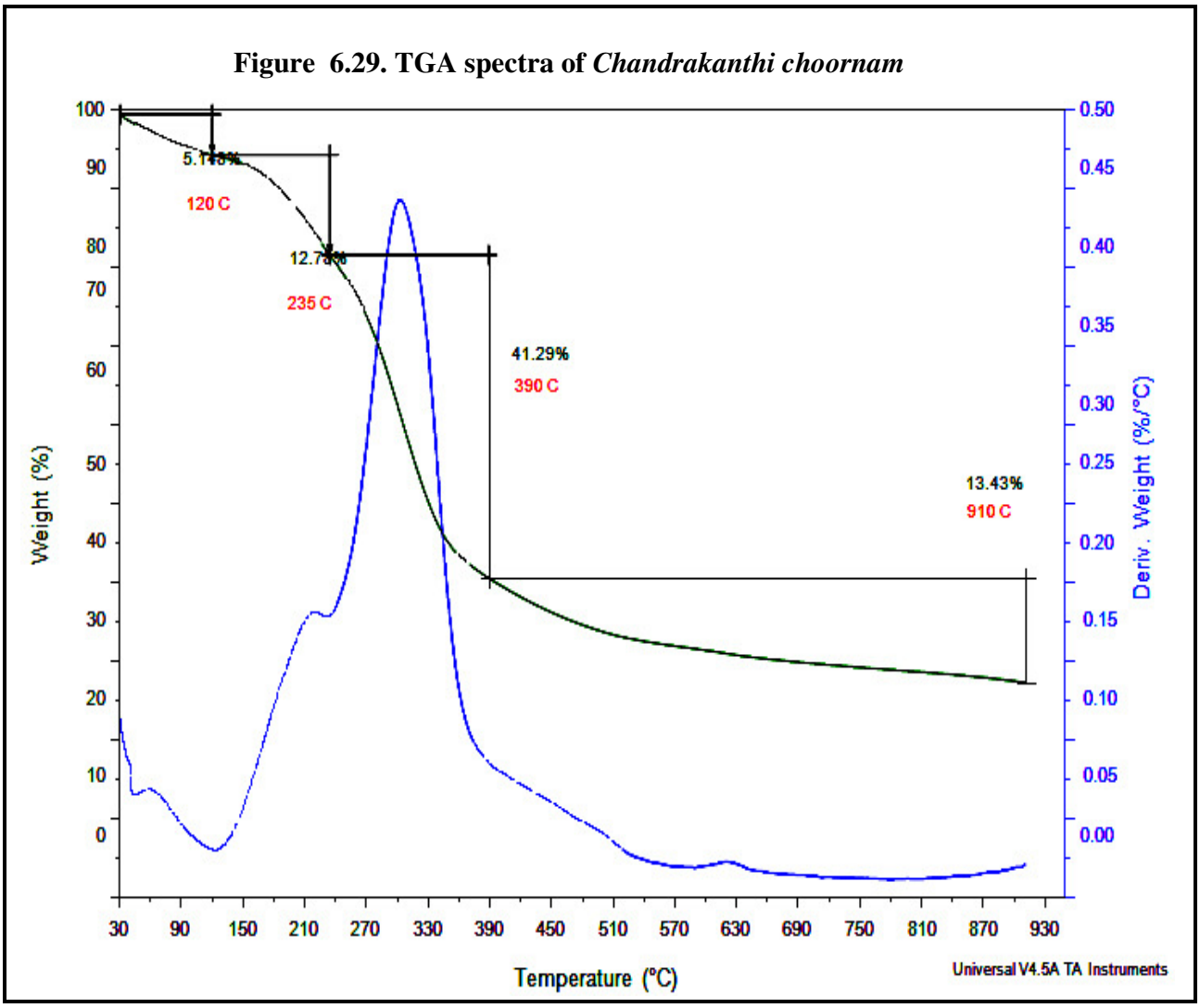
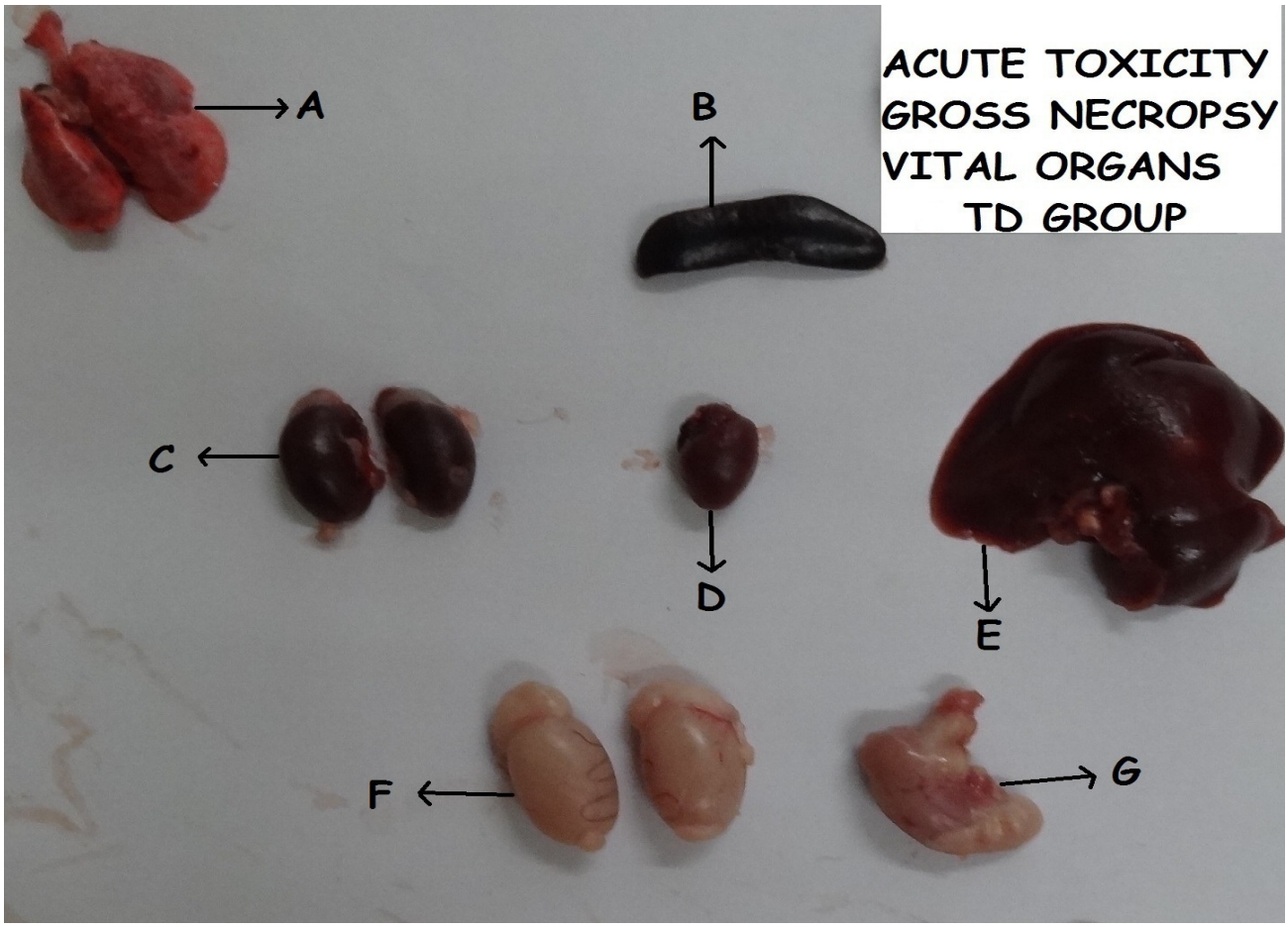


Figure 6.30A: Gross necropsy of the vital organs in acute toxicity study

Normal Control **Vehicle Control** **10 TD**

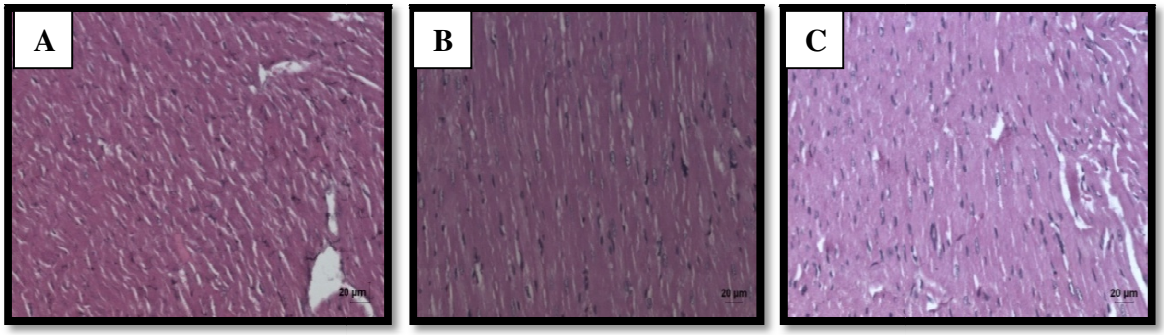
Figure 6.30B: Gross necropsy of the vital organs in acute toxicity study (10 TD Group)



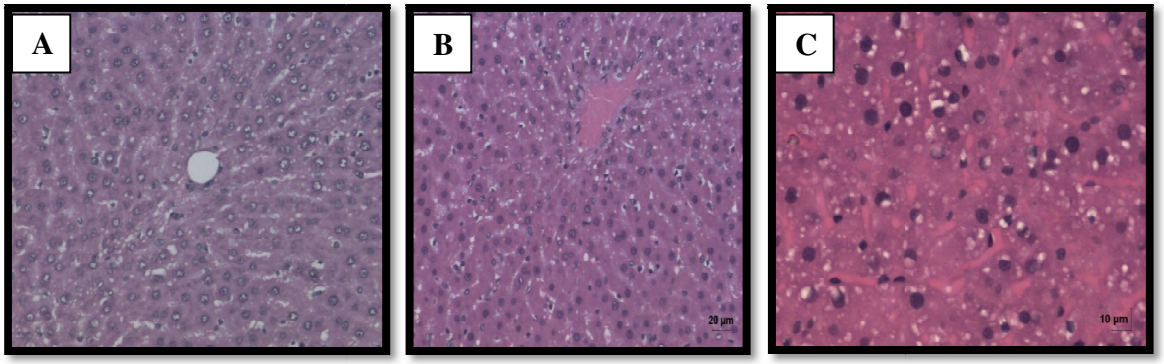
A- Lungs; B- Spleen; C- Kidney; D- Heart; E- Liver; F- Testis; G- Caecum

Figure 6.33: Histopathological photomicrographs of rats in longterm toxicity study

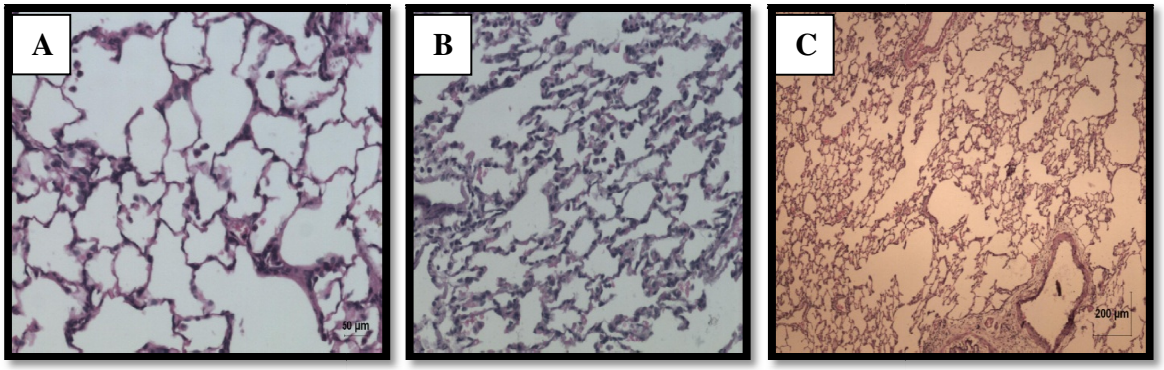
HEART



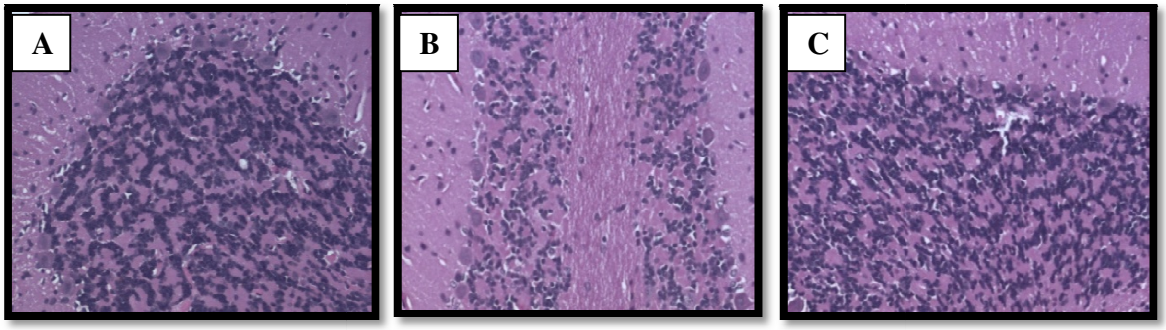
LIVER



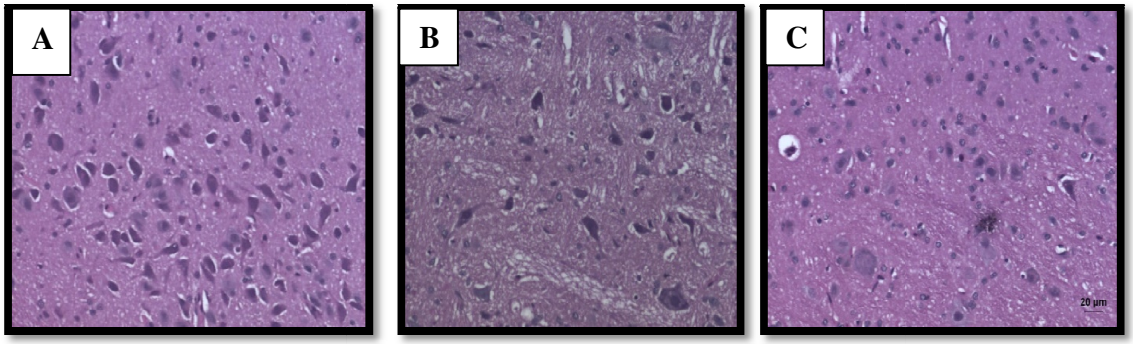
LUNGS



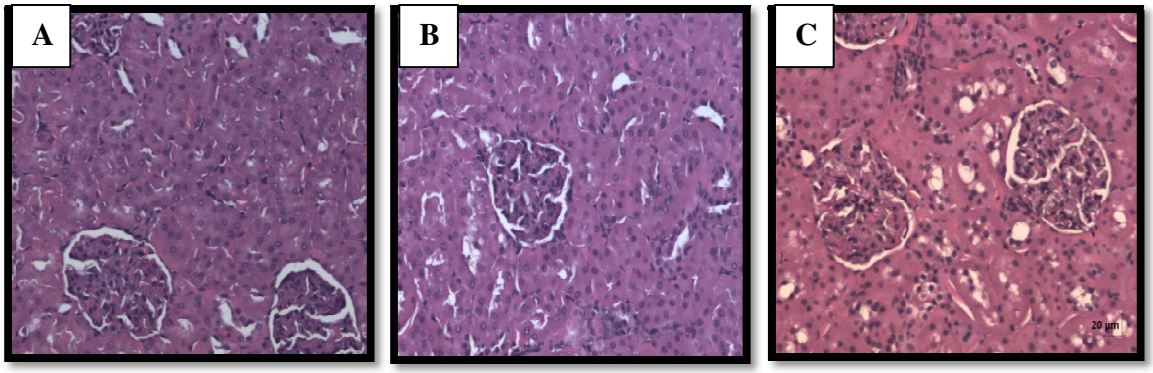
CEREBELLUM



CEREBRUM



KIDNEY



SPLEEN